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**The American Physiological Society**

BY

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THE DIASTASE IN CAT'S SALIVA.

BY A. J. CARLSON AND J. G. RYAN.

[From the Hull Physiological Laboratory, the University of Chicago.]

IT is commonly held that the saliva of the carnivora in general contains no ptyalin or starch-splitting ferment. This has been affirmed by many investigators, particularly for the dog. The only results to the contrary appear to be those recently reported by Nielson and Terry.<sup>1</sup> Less work appears to have been done on the cat's saliva, but the references to the subject that we have been able to find in the literature indicate that the cat's saliva is also devoid of starch-splitting ferments.

The cat is, however, a relatively inexpensive laboratory animal, and very serviceable for the collection of saliva both from the parotid and the submaxillary glands. We therefore made some preliminary tests of the diastatic power of cat's saliva in our search for a suitable laboratory animal for the study of some points in the secretion of ptyalin. As our results were positive, the experiments were extended; but we soon discovered that while the cat's saliva contains a starch-splitting ferment, this ferment is not a specific product of the salivary gland, but the diastase of the blood carried into the saliva along with other salivary constituents. The cat cannot therefore be used for the study of the conditions of secretion of ptyalin. But the condition here found in the cat is, as far as we know, unique for the salivary glands, and merits further investigation.

<sup>1</sup> NIELSON and TERRY: This journal, 1906, v, p. 406.

## I. THE LITERATURE.

Nielson and Terry report the presence of diastase in the dog's mixed saliva and in the aqueous extracts of the dog's salivary glands. They were also able to demonstrate an increase in the percentage of diastase in the saliva of dogs fed exclusively on carbohydrates. At the meeting of the American Physiological Society in New York, 1906, Nielson reported further results of similar experiments, starch-splitting ferments being found in the saliva in a few of the dogs only. Mendel and Underhill<sup>2</sup> repeated the experiments of Nielson and Terry with uniformly negative results. Neither on carbohydrate nor on meat diet did the dog's saliva digest starch, according to their report. They call attention to the positive and definite conclusions drawn by Bidder and Schmidt, Claude Bernard, and Starling as to the absence of diastase in dog's saliva. They seem, however, to restrict the term "amylolytic activity" to those ferments which carry the starch beyond the dextrine stage to maltose.

Cannon and Day,<sup>3</sup> in a review of the literature, make mention of the work of Brücke, V. Mering, and Seegen, who were unable to demonstrate any amylolytic action in the stomach of dogs fed upon a carbohydrate diet (starch), their conclusions being that the dog's saliva does not possess a diastatic ferment.

The saliva of the cat has received much less attention, and so far as we know there has been no positive demonstration of a diastatic ferment in the saliva of the cat. Latimer and Warren<sup>4</sup> extracted an amylolytic zymogen from the salivary glands of the dog, cat, sheep, and ox. They made extracts of the various glands with chloroform or 1 per cent sodium fluoride, activating them for ten minutes with 1 per cent acetic acid, then neutralizing and testing for digestive power. But these results prove nothing as regards the presence of ptyalin in the cat's saliva, because of the presence of amylases in the tissues, the blood, and lymph.

Cannon and Day found great digestion of starch in the stomach of cats fed through a tube with crackers mixed with human saliva. They also made control tests by allowing the cat to eat dry crackers, and found only slight traces of sugar in the stomach after one half

<sup>2</sup> MENDEL and UNDERHILL: *Journal of biological chemistry*, 1907, iii, p. 135.

<sup>3</sup> CANNON and DAY: *This journal*, 1903, ix, p. 396.

<sup>4</sup> LATIMER and WARREN: *Journal of experimental medicine*, ii, p. 465.

to two hours. This small amount of reducing substance may have been due to possible traces of sugar in the food, to a feeble diastatic action, or to the sugar present in the cat's saliva.<sup>5</sup> In their report they make no statement as to the presence or absence of an amylolytic ferment in the cat's saliva, but we assume from their control test that they consider the cat's saliva to have no marked diastatic power.

## II. METHODS.

Large cats fed upon a mixed diet of bread and meat were selected for the experiments. In a few cases, however, the diet was unknown.

The technique of the experiments was as follows:

1. In four experiments mixed saliva was collected reflexly from the mouth after previously washing by stimulating the mucous membrane with vapor of ether or dilute acetic acid.

2. In two experiments temporary fistulæ were made by placing cannulas in Stenson's ducts under local anæsthesia (ethyl chloride), and parotid saliva collected reflexly as mentioned above.

In these experiments the cats were held by placing the neck in a stock.

3. In nine experiments the cats were anæsthetized and cannulas placed in Wharton's ducts and Stenson's ducts, and successive samples of 1 c.c. each of submaxillary saliva collected by stimulation of the chorda tympani. Usually one or two samples were obtained at the same time from the parotid by spontaneous flow (reflex). After collecting two to four chorda samples and one or two parotid samples, the diastatic power of the blood was then increased by injection of (a) human saliva (three experiments), (b) commercial pancreatin (three experiments), and (c) malt diastase (three experiments). Then, on fatigue of the chorda tympani, pilocarpine was injected either intravenously or directly into the muscles of the leg. By this procedure several more samples of submaxillary saliva could be obtained simultaneously with those from the parotid.

The various injections mentioned above were as follows:

- a. Mixed human saliva was filtered and 10 to 15 c.c. injected intravenously.

<sup>5</sup> MENDEL and UNDERHILL: *Loc. cit.*; CARLSON and RYAN: This journal, 1908, xxi, p. 301.

b. In the case of the commercial pancreatin 10 to 15 c.c. of a 4 per cent in 0.95 per cent salt solution was injected very slowly, owing to the presence of peptone. Then, after collecting two or more samples of saliva, this injection was followed by injection of 10 to 15 c.c. of human saliva.

c. In three separate experiments the procedure in (b) was repeated, using 10 c.c. of a 6 per cent solution of malt diastase instead of commercial pancreatin.

4. In the last experiments, of which there were six, alternating samples of chorda and sympathetic submaxillary saliva were collected by alternate stimulation of the chorda tympani and cervical sympathetic. In order to be sure that the alternating samples were pure chorda and pure sympathetic, the cannulas were always carefully emptied with a Pasteur pipette, and then the first two drops discarded so as to get rid of what remained in the ducts after each sample.

Most of the experiments were conducted under as nearly sterile conditions as possible. The ducts after being isolated were always carefully washed with physiological salt solution to remove any blood or serum that had collected on them, the duct opened with sterile scissors, and the various samples of saliva drawn through sterile cannulas into sterile graduates. The starch tubes were always sterilized, and the starch emulsion kept as free from bacteria as possible by plugging the tubes with cotton. In a few experiments the only precaution used was addition of thymol to the starch emulsion.

Two or three experiments were carried out without these precautions in order to test whether sterile conditions would influence the results. The results of those experiments carried on in the most careful manner were in every detail identical with those in which no precautions at all were taken.

The receptacles for the saliva were kept well corked to prevent evaporation and concentration.

The various samples of saliva, after being carefully equalized to 1 c.c. each, were added to 5 c.c. of a 1 per cent solution of arrow-root starch. In part of the experiments the digestive tests were made at room temperature and part at 37° C. Parallel tests were made of the serum from each animal.

After defibrination and centrifugalization of the blood, varying quantities of the serum — one drop up to 1 c.c. — were added to



a series of starch tubes and the digestive test carried on simultaneously with those of the saliva. Care was taken to have everything in readiness, so that the various samples of saliva and serum could be added to the starch tubes at as nearly the same moment as possible. In those cases in which the concentration of the diastase of the blood was increased by the injection of the above-named ferments, parallel determinations were made with both the serum and urine before and after the injection.

After allowing the starch saliva tubes to stand for some time, the interval varying with the temperature, the tubes were examined, (a) by noting the degree of clearing of the boiled starch solution, and (b) by testing for the dextrines with iodine. We made use of these methods rather than the determination of the sugars formed, inasmuch as the cat's saliva itself contains glucose in varying amounts. That method would have rendered it necessary to determine the amount of sugar in each sample of saliva.

### III. RESULTS.

1. The normal or reflex mixed saliva of the cat contains a starch-splitting ferment or ferments capable of clearing a boiled starch solution and carrying the hydrolysis at least to the archodextrine stage. In no case did we fail to get clearing of the solution. This clearing was not due to contamination with bacterial ferments from the mouth, because the mouth was always carefully washed with warm water prior to the experiment. The possibility of such contamination is, furthermore, excluded by collecting the saliva by means of a temporary fistula. When asepsis is maintained in this case, the clearing of the starch solution cannot be ascribed to the growth of bacteria in the digestion tube itself. We are thus forced to the conclusion that the normal cat's saliva contains a ferment or ferments capable of hydrolyzing starch. The concentration of this ferment varies in saliva from different animals. On the whole, the amount of this ferment in the saliva is exceedingly small in comparison with that in human saliva. There is nothing to show that this diastatic ferment in the cat's saliva is identical with ptyalin.

2. *The concentration of the diastase is invariably greater in the reflex saliva or the chorda or pilocarpine saliva collected under general ether anæsthesia than in the reflex saliva collected without*

*anæsthesia*. This difference is considerable, although we are not in position to express it in figures. The difference cannot be due to the dilution of the normal saliva by the secretions from the glands in the mucous membrane of the mouth itself, because it is obtained even when the parotid saliva is secured from a temporary fistula. The probable explanation of this difference will be considered in connection with the source of the ferment. A typical experiment demonstrating this difference is given in Table I.

TABLE I.

Detail of part of one experiment on the diastase of the cat's saliva, showing greater concentration of the diastase in the saliva collected under general ether anæsthesia than in normal or reflex saliva. Digestion at 37° C.

Saliva.	Amt. of saliva used.	Amt. of starch used.	Time.		
			1 hour.	1½ hours.	2 hours.
1 Reflex mixed . . . .	e.e. 1	e.e. 5	No action.	Clear.	....
2 { Chorda from same cat under ether anæsthesia }	1	5	Clear.	....	....
1 { Reflex parotid (tempo- rary fistula) . . . . }	1	5	No action.	No action.	Partly clear.
2 { Sample collected by pil- ocarpine injection (ether anæsthesia) . }	1	5	No action.	Nearly clear.	Clear.

3. *The concentration of the diastase in the submaxillary saliva is greater than in the parotid.* It at once became evident that such a difference existed, for in every experiment in which the two salivas were compared the submaxillary starch-saliva tubes always were first to show evidence of clearing of the starch emulsion and the arrival of the erythro-dextrine. And the difference is so considerable that there is no possibility of ascribing it to experimental errors. Such errors, moreover, could not always work in one direction. The cat's parotid is a serous gland, while the submaxillary is mainly if not wholly mucous. This being the case, our findings appear to be unique, and contrary to the usual fact that the serous salivary glands are the ptyalin producers, while the mucous-forming gland secretes little or no diastase.

The relative concentration of the diastase in the cat's submaxil-

lary and parotid saliva thus exhibits the same condition as that of the glucose in these salivas.<sup>6</sup> The same cause or causes are probably responsible for both phenomena, but at present we can offer no satisfactory explanation of either.

The details of one typical experiment showing this fact are given in Table II.

4. *The successive samples of saliva collected during a single period of gland activity show a gradual diminution in the concentration of the starch-splitting ferment.* This is particularly evident in the submaxillary gland saliva, and we paid particular attention to this point in that gland. After a period of rest of the gland, the saliva becomes again richer in diastase. This fact of gradual diminution in the diastase is apparent on examination of the submaxillary saliva series (I and III) in Table II.

The obvious explanation of this phenomenon is the gradual diminution in the prozymogen of the gland during the activity, that is, the simple phenomenon of gland fatigue. But the diminution in the concentration of the diastase certainly appears sooner than the appearance of any microchemical sign of fatigue in the gland cells. The percentage of the organic solids in the saliva exhibits the same gradual diminution during a simple period of activity, as shown originally by Ludwig and more recently for the cat's saliva by Carlson and McLean.<sup>7</sup> With the exception of mucin and possibly ptyalin, there is no evidence that these organic constituents are specific products of the gland cells. In fact, it has been shown by us that it is not the case of the sugar in the cat's saliva. The sugar is the glucose of the blood eliminated into the saliva. The diminution of any constituent of the saliva during activity is therefore no evidence of gland fatigue or of the specificity of these constituents for that gland. It is possible, for example, that the organic constituents of the blood and lymph reach and enter the gland cells more slowly than do the water and salts. Some of the organic constituents of the blood and lymph may enter the gland cells and be eliminated in the saliva. After a period of rest of the gland these substances would be present in the gland cells in greater concentration than after a period of activity, and may therefore be eliminated with the saliva in greater abundance at first. If this is the correct explanation of the gradual

<sup>6</sup> CARLSON and RYAN: *Loc. cit.*

<sup>7</sup> CARLSON and MCLEAN: This journal, 1908, xx, p. 457.

TABLE II.

Detail of one experiment on the relative concentration of diastase in the parotid and submaxillary saliva of the cat. Showing the greater concentration in the submaxillary saliva. Digestion at 37° C.

Saliva samples.	Amt. of saliva used.	Amt. of starch used.	Time.				Iodine.	
			1½ hrs.	2½ hrs.	3½ hrs.	4 hrs.	4½ hrs.	5 hrs.
I. Right submaxillary.	c.c.	c.c.						
1. Chorda stim. . .	1	5	Clear.	....	....	....	Red.	No color.
2. Chorda stim. . .	1	5	Clear.	....	....	....	Red.	No color.
3. Chorda stim. . .	1	5	Partly clear.	Clear.	....	....	Red.	Red.
4. Chorda stim. . .	1	5	No clearing.	Nearly clear.	Clear.	....	Red.	Red.
5. Pilocarp. inj. . .	1	5	No clearing.	No clearing.	Clear.	....	Tinge of red.	Red.
6. Pilocarp. inj. . .	1	5	No clearing.	No clearing.	Clear.	....	Tinge of red.	Red.
II. Right Parotid.								
1. Spontaneous flow	1	5	No clearing.	No clearing.	Partly clear.	Clear.	Blue.	Blue.
2. After pilo. inj. .	1	5	No clearing.	No clearing.	Partly clear.	Clear.	Blue.	Blue.
3. After pilo. inj. .	1	5	No clearing.	No clearing.	Partly clear.	Clear.	Blue.	Blue.
III. Left submaxillary.								
1. Chorda stim. . .	1	5	Clear.	....	....	....	Red.	No color.
2. Chorda stim. . .	1	5	Clear.	....	....	....	Red.	No color.
3. Chorda stim. . .	1	5	Clear.	....	....	....	Red.	Tinge of red.
4. Chorda stim. . .	1	5	Partly clear.	Clear.	....	....	Tinge of red.	Red.
5. Chorda stim. . .	1	5	No clearing.	Clear.	....	....	Tinge of red.	Red.
6. Pilocarp. inj. . .	1	5	No clearing.	Partly clear.	Clear.	....	Tinge of red.	Red.
7. Pilocarp. inj. . .	1	5	No clearing.	Partly clear.	Clear.	....	Blue.	Tinge of blue.
IV. Left parotid.								
1. Spontaneous flow	1	5	No clearing.	No clearing.	Nearly clear.	Clear.	Blue.	Blue.
2. Pilocarp. inj. . .	1	5	No clearing.	No clearing.	Nearly clear.	Clear.	Blue.	Blue.
3. Pilocarp. inj. . .	1	5	No clearing.	No clearing.	Nearly clear.	Clear.	Blue.	Blue.

diminution of the salivary diastase, we should expect to find a greater concentration of the diastase in the saliva collected during partial anæmia of the gland, as it has been shown recently by Carlson, Greer, and Becht, and by Carlson and McLean<sup>8</sup> that

TABLE III.

Details of one experiment showing the greater concentration of the diastase in the sympathetic than in the chorda submaxillary saliva. Digestion at 37° C.

Saliva.	Amt. of saliva used.	Amt. of starch used.	Time.			Iodine.	
			1½ hours.	2½ hours.	3½ hours.	4 hours.	5 hours.
I. R't submaxillary							
1. Chorda	1	5	Nearly clear.	Clear.	....	Red.	No change in any of the tubes.
2. Sympathetic	1	5	Nearly clear.	Clear.	....	Red.	
3. Chorda	1	5	No action.	Partly clear.	Clear.	Tinge of red.	
4. Sympathetic	1	5	Partly clear.	Clear.	....	Red.	
5. Chorda	1	5	No action.	Slight action.	Clear.	Blue.	
II. L't submaxillary							
1. Sympathetic	1	5	Clear.	....	....	Red.	Practically no change in any of the tubes.
2. Chorda	1	5	No action.	Partly clear	Clear.	Bluish.	
3. Sympathetic	1	5	Clear.	....	....	Red.	
4. Chorda	1	5	No action	No action	Clear.	Bluish.	
5. Sympathetic	1	5	Clear.	....	....	Red.	
6. Chorda	1	5	No action	No action	Partly clear.	Blue.	

partial anæmia increases the percentage of the organic constituents of the saliva by diminishing the rate of secretion of the water and the inorganic salts.

5. When alternate samples of saliva from the submaxillary gland or chorda and on sympathetic stimulation are tested for diastatic power, the sympathetic saliva almost invariably shows the greater concentration of the ferment. The experiment can be repeated several times on the same gland, and of all the animals tested on

<sup>8</sup> CARLSON, GREER, and BECHT: This journal, 1907, xx, p. 180; CARLSON and MCLEAN: *Loc. cit.*, p. 457.

this point the results were positive, but the difference not always equally marked. Results like these may be obtained if two or three chorda samples are taken before the sympathetic sample: the sympathetic saliva may show less diastatic power than the first and second samples of the chorda saliva, although it has a stronger action than the sample of chorda saliva immediately preceding it. But this is in reality no exception to the law as stated. It simply means that the chorda saliva may be richer in diastase than the sympathetic saliva from the same gland, in case the two salivas are collected at different stages in the activity of the gland. Given the condition of the gland as nearly the same as possible, the sympathetic saliva contains the greater percentage of diastase.

This fact is easily demonstrated, but the explanation of it not so readily found. It will be recalled that the chorda and the sympathetic submaxillary saliva of the cat show similar differences in the percentage of glucose, but not in the total organic solids. When the cause of this difference in the organic solids is found, we shall in all probability have the explanation of the difference in the concentration of the diastase.

The reader is referred to Table III for an illustration of this difference in the concentration of the diastase in chorda and sympathetic saliva.

6. *The starch-splitting enzyme or enzymes are very much more concentrated in the cat's serum than in the cat's saliva.* The relatively weak diastatic action of the cat's saliva suggested the possibility that the ferment in the saliva may not be a specific product of the salivary glands, but simply the blood and lymph diastase transferred into the saliva along with other serum and lymph constituents. To test this possibility we made numerous parallel tests of the relative diastatic power of the serum and the saliva from the same animal, and in every case the serum showed a very much greater rate of action. For example, 1 c.c. of serum added to 5 c.c. of the 1 per cent boiled starch solution rendered the starch solution clear in about five minutes even at the room temperature, while the same proportions of saliva and starch take from one and one half to two hours to effect complete clearing even at 37° C. There are some variations in the diastatic power of the blood from different cats, but in no case did we find the diastatic power of the saliva even approximate that of the serum.

It is well known that human parotid saliva has a very much

greater power than has the human serum. One series of parallel tests of the diastatic power of the parotid saliva and the serum was made on material from one of the authors (A. J. C.). The human serum showed about the same diastatic power as the cat's

TABLE IV.

Detail of one series of experiments on the relative diastatic action of cat's serum and cat's saliva. Digestion at 37° C.

Serum series.	Amt. of serum used.	Amt. of starch used.	Time.				Iodine.	
			15 min.	30 min.	45 min.	1 hr.	1 hour 15 min.	2 hrs.
I. 1	drop 1	c.c. 5	No action.	Clear.	....	....	Blue.	Blue.
2	2	5	" "	"	....	....	Blue.	"
3	3	5	Clear.	....	....	....	Blue.	Red.
4	4	5	"	....	....	....	Blue.	"
5	5	5	"	....	....	....	Red.	No color.
6	6	5	"	....	....	....	"	" "
7	7	5	"	....	....	....	"	" "
8	8	5	"	....	....	....	No color.	" "
9	9	5	"	....	....	....	" "	" "
10	10	5	"	Blue (iodine).	Red (iodine).	No color (iodine).	" "	" "

Serum.	Amt. of serum used.	Amt. of starch used.	Time 2.52 P. M.	Iodine.	
				3.30	4.45
II.	c.c. 1	c.c. 5	Clear in 5 min.	Red (iodine).	No color (iodine).
III.	1	5	Clear in 5 min.	Red (iodine.)	No color (iodine).

serum, while the starch-splitting action of the human parotid saliva was incomparably greater.

The relatively small amount of the diastase in the cat's saliva and the further fact that the cat's serum contains diastase in much greater concentration than does the saliva support the view that

the cat's salivary diastase is not a product of the salivary glands, but simply a portion of the blood and lymph diastase passed into the saliva along with other constituents. On this view the cat's salivary glands are not special makers of ptyalin. If the hypothesis is correct, it ought to be possible to increase the percentage of the diastase in the saliva by increasing it in the serum and lymph. Experiments are in progress in the laboratory with the view of finding some method of increasing the concentration of the diastase normally present in the serum, but at present no such means are available. We were therefore obliged to increase the diastatic power of the serum and lymph by intravenous injection of foreign starch-splitting ferments. The ferments employed were human ptyalin (mixed saliva), pancreatic amylopsin (commercial pancreatin), and malt diastase.

*7. Intravenous injection of mixed human saliva and of pancreatin increases the diastatic power of cat's saliva, but similar injection of malt diastase has no effect on the concentration of the salivary diastase.* The increase of salivary diastase was always more marked after injection of human saliva than after the injection of pancreatin. And inasmuch as the saliva injected always had a stronger diastatic action than the solutions of pancreatin used, it would seem that the greater the concentration of the starch-splitting ferments in the blood and lymph, the greater the fraction of them passing into the saliva. While the injection of saliva and pancreatin increase the diastatic action of the saliva subsequently collected, the salivary diastase never becomes as concentrated as that of the serum.

We were not a little surprised at the negative results of the experiments with malt diastase. Apparently that ferment does not readily pass into the salivary gland cells and the saliva. The negative results are not due to the rapid destruction of the diastase in the blood or its elimination from the blood. While it is partly eliminated by the kidneys, yet its disappearance from the blood is so gradual that its presence in considerable concentration can be shown for at least one and one half hours after the injection.

The fact that human ptyalin and amylopsin pass through the salivary glands while malt diastase does not seems to indicate that these ferments are structurally different.

We admit that these experiments with the injection of human



ptyalin and pancreatic amylopsin do not constitute a demonstration of our hypothesis that the salivary diastase in the cat is simply the diastase of the blood and lymph passed into the saliva along with other blood and lymph constituents. Human ptyalin

TABLE V.

Details of one series of experiments showing the increase in the diastatic power of cat's saliva after intravenous injection of human saliva and commercial pancreatin. Digestion at 37° C.

Saliva.	Amt. of saliva used.	Amt. of starch used.	Time.		Iodine.	
			1½ hrs.	2¼ hrs.	3 hrs.	4 hrs.
I. Right submaxillary.	c.c.	c.c.				
1. Chorda before inj. of pancreatin	1	5	Nearly clear.	Clear.	Tinge of red.	Red.
2. Chorda before inj. of pancreatin	1	5	Slight clearing.	Clear.	Blue.	Red.
3. Pilocarp. before inj. of pancreatin	1	5	No action.	Partly clear.	Blue.	Bluish.
4. Pilocarp. after inj. of pancreatin	1	5	No action.	Partly clear.	Blue.	Bluish.
5. Pilocarp. after inj. of pancreatin	1	5	Partly clear.	Clear.	Tinge of red.	Red.
6. Pilocarp. after inj. of pancreatin	1	5	Nearly clear.	Clear.	Tinge of red.	Red.
7. Pilocarp. after inj. of human saliva	1	5	Clear.	....	Red.	No color.
8. Pilocarp. after inj. of human saliva	1	5	Clear.	....	Red.	No color.
II. Right parotid.						
1. Pilocarp. before inj. of pancreatin	1	5	No action.	Slight action.	Blue.	Blue.
2. Pilocarp. before inj. of pancreatin	1	5	No action.	Slight action.	Blue.	Blue.
3. Pilocarp. after inj. of pancreatin	1	5	No action.	Slight action.	Blue.	Blue.
4. Pilocarp. after inj. of pancreatin	1	5	Slight action.	Clear.	Tinge of red.	Red.
5. Pilocarp. after inj. of human saliva	1	5	Clear.	....	Red.	No color.
6. Pilocarp. after inj. of human saliva	1	5	Clear.	....	Red.	No color.

is a foreign substance to the cat's blood, and as such may be eliminated in the saliva as well as by the kidney just as happens to many other foreign substances introduced into the blood. The point cannot be proven absolutely before we are able to vary the concentration in the blood of the starch-splitting ferments nor-

mally present there. All the facts so far as known, however, speak for the correctness of our hypothesis. These facts are:

(1) The much greater concentration of the diastase in the blood than in the saliva.

(2) The increase in the salivary diastase by the intravenous injection of human ptyalin and pancreatic amylpsin.

(3) The direct variation of the concentration of the salivary diastase with the concentration of the organic solids in the saliva.

(4) The greater concentration of the diastase in the submaxillary than in the parotid saliva.

(5) The fact that extracts of the cat's salivary glands have a diastatic action is, of course, no proof that the cat's salivary glands are special producers of the starch-splitting ferments as compared to the tissues in general. Starch-splitting ferments are found in the blood and the lymph as well as in all of the tissues. These ferments are either formed in small amounts in special tissues, in which case they are taken up by other organs along with other constituents of the blood, or they are formed in all tissues as necessary elements in their metabolism.

8. We have made a few experiments of the diastatic action of the dog's saliva in order to determine whether the conditions obtained in the cat are not also present in the dog. This appears to be the case. In only two experiments did we find any starch-splitting action in the dog's saliva, and even in these two instances the dog's serum showed much greater diastatic power than the saliva, just as in the case of the cat. It is therefore highly probable that the dog's salivary glands do not produce any starch-splitting ferments, but that the slight diastatic action of dog's saliva, occasionally found, is due to the blood and lymph diastase transferred into the saliva along with other constituents. The two factors determining the presence or absence of the diastase in the cat's and the dog's saliva are obviously (1) the concentration of the diastase in the blood and lymph, (2) the condition of the salivary glands.

Inasmuch as the dog's salivary glands do not secrete starch-splitting ferments in the sense that this is done by the parotid glands of man and the herbivora in general, one seems to be forced to the conclusion that the only basis for the results of Nielson and Terry is some error of observation. Still, it is possible that a carbohydrate diet may increase the percentage of the starch-

splitting ferments in the blood and lymph; in which case their observations may be correct, although their interpretation of them is not. This possibility seems remote, in view of the negative results of the work of Mendel and Underhill, but the question is now being investigated in our laboratory.

# THE INFLUENCE OF SALTS AND NON-ELECTROLYTES UPON THE HEART.

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## INTRODUCTION.

THE current theories regarding the relations of inorganic salts and non-electrolytes to heart beat may be summarized as follows:<sup>1</sup> According to Howell, the stimulus to initiation and maintenance of heart beat is furnished by the cations contained in the blood. This writer lays especial emphasis upon calcium ions as the stimulating agent. Potassium ions are considered to be inhibitory in their action upon heart tissue, and their function is to counterbalance the too strongly stimulating effects of calcium ions. Sodium ions are also essential for heart rhythm, but their function is chiefly to maintain proper osmotic pressure. Non-electrolytes are regarded as having no direct stimulating action upon heart tissue.

Lingle<sup>2</sup> has opposed Howell's view that calcium ions constitute the stimulating agent for heart tissue, and ascribes this function to the sodium ions. In accordance with Loeb's view, he maintains that pure solutions of sodium salts are poisonous to heart tissue, and that the function of potassium and calcium ions is largely to neutralize this poisonous effect.

Langendorff<sup>3</sup> has presented the hypothesis that heart tissue derives the stimulus to rhythmic activity from the products of its own metabolic activity rather than from the salts of the blood.

<sup>1</sup> For the extensive literature on this subject consult HOWELL: This journal, 1898, ii, p. 47; GREEN: *Ibid.*, p. 82; and LANGENDORFF: *Ergebnisse der Physiologie*, 1902, part 2, p. 264, where references are given to work reported up to 1902. Results published since that time which have a bearing upon our present discussion will be considered individually.

<sup>2</sup> LINGLE: This journal, 1900 iv, p. 265; *Ibid.*, 1902, viii, p. 75.

<sup>3</sup> LANGENDORFF: *Loc. cit.*

Martin <sup>4</sup> has recently presented evidence which he believes lends experimental support to Langendorff's hypothesis. From his experimental data Martin assumes that the metabolic products, which in accordance with Langendorff's hypothesis are regarded as furnishing the stimulus to rhythmic activity, are mainly, if not entirely, formed as the direct result of oxidative processes. He therefore maintains that the chief factor in initiation and maintenance of heart beat is proper oxidation within the tissue. To calcium ions is assigned the function of promoting this oxidation.

In a previous paper <sup>5</sup> the present writer suggested that the tonus of heart tissue is an important factor in determining the response of cardiac tissue to stimuli, and further pointed out that the anion probably plays a positive rôle in the production of phenomena which follow the application of salt solutions to heart tissue. In the present paper further evidence will be presented that there are two factors involved in the production of heart rhythm,—the tonus of the tissue, and the stimulating agent; and a brief critique will be offered of conclusions arrived at by other investigators. In all the experiments described, strips of the turtle's ventricle prepared as described by Green <sup>6</sup> have been used.

#### THE INITIATION OF THE BEAT.

A fresh strip of turtle's ventricle immersed in the animal's own serum or in Ringer's solution does not give a series of beats. Substituting for the serum or Ringer's a 0.7 per cent solution of sodium chloride, a very different result is obtained. After a shorter or longer period of time, termed the "latent period," the strip begins to beat, and gives a regular series of contractions lasting usually from one to three hours.<sup>7</sup> These two facts, brought out years ago, should form a basis for the interpretation of all heart-beat phenomena.

Until very recently the method of producing beats in a fresh strip with sodium chloride was regarded as the only way of obtaining beats from such a strip. Yet an experiment described some

<sup>4</sup> MARTIN: This journal, 1905-1906, xv, p. 303; *Ibid.*, 1906, xvi, p. 191.

<sup>5</sup> BENEDICT: This journal, 1905, xiii, p. 192.

<sup>6</sup> GREEN: This journal, 1898, ii, p. 82.

<sup>7</sup> MERUNOWICZ: *Arbeiten aus dem physiologischen Anstalt zu Leipzig*, 1875, p. 132.

years ago by Howell,<sup>8</sup> which in the present writer's opinion was never properly interpreted by Howell or other writers on this subject, furnishes, by a slight modification, a method of producing beats in what is practically a fresh strip, and contributes an important factor to the theory of heart beat. Howell immersed a fresh heart strip in sodium oxalate solution for a short time, after which treatment it was immersed in sodium chloride solution. No beats developed in this latter solution, even after prolonged immersion. Removal to a Ringer's solution was followed shortly afterwards by a series of beats. Howell interpreted this result to indicate that calcium is essential to the production of heart beat. If we modify the procedure slightly, and lay emphasis upon another aspect of the experiment, a very interesting result is obtained. A fresh strip of turtle's ventricle is immersed for a few (five) minutes in 1 per cent sodium or ammonium oxalate solution, after which treatment it is transferred directly to Ringer's mixture. A series of contractions begins almost at once, which may last for from one to three hours. The significant facts here, and those which Howell failed entirely to bring out, are as follows: *A fresh strip, which, as will be remembered, will not beat in Ringer's solution, can be made to give an immediate series of contractions in this calcium-containing medium by previous treatment with a calcium-removing agent (oxalate).* Since the immersion in oxalate is too brief to permit loss of a significant quantity of potassium ions, and since the subsequent beats in Ringer's solution begin practically at once, we must infer that Howell's hypothesis that potassium ions constitute the inhibitory factor in ventricular tissue is at least partially incorrect, and we must further conclude that *removal of calcium ions is, in this instance at least, the essential factor in enabling the tissue to respond with rhythmic contractions to subsequent treatment with Ringer's solution.* To offer an explanation of these results which is based upon the presence or absence of any ions within the tissue would appear very difficult. There is, however, a marked change which occurs in the tissue upon treatment with oxalate solution, and it is upon this change that the writer bases his interpretation of the experiment. As a result of the application of oxalate solution a *loss of tonus* takes place in the heart strip. If, then, we assume that the fresh strip is in too great a state of tonus to permit of its giving a rhythmic

<sup>8</sup> HOWELL: This journal, 1901-1902, vi, p. 192.

series of beats, we should expect that temporary removal of calcium ions (which ions strongly increase tonus in heart strips) would be followed by a series of beats in Ringer's solution, since this solution contains the proper ions for rhythmic activity, and in it the too great tonus-increasing power of the calcium ions is counterbalanced by potassium ions. Since, in this experiment at least, the only change which we can detect in the heart strip, as it is becoming irritable (if we may use the expression) to Ringer's solution, is a tonus change, we may conclude that a heart strip can be brought into a condition where beats can be produced by agents previously ineffective, by means of changes brought about in the tissue's irritability. These changes in irritability may be indicated by tonus changes occurring in the muscle. This position is substantially that proposed in a previous paper,<sup>9</sup> where it was suggested that the chief function of the cation in relation to heart activity may be the preservation of the tissue in a condition of tonus best suited to its activity.

In a recent paper Martin<sup>10</sup> has criticised the present writer's point of view in regarding tonus as an essential factor in the determination of the response of heart tissue to stimuli, stating that he (Martin) finds it "easier to look upon the tone changes which accompany the responses of heart strips to various treatments as in the nature of effects rather than causes." By the statement made in this connection the present writer did not intend to imply that tonus as such is a causative factor. The writer's position regarding this point may be briefly stated as follows. It is a well-known fact that skeletal muscle in one condition of tonus is a very different tissue as regards its power of responding to stimuli from the same tissue in another condition of tonus. Skeletal muscle in a relaxed condition is not so responsive to stimuli as when in a slight degree of tonus. As the tonus increases (for example, in a muscle passing into *rigor caloris*), the tissue may become exceedingly irritable, stimuli frequently producing several contractions, which under other conditions are unable to produce even one. In its greatest degree of tonus (*rigor caloris*) the muscle is incapable of giving any response to stimuli. These tonus changes are not *per se* causative, but they may represent the condition of the tissue as regards its irritability. Furthermore, it is to be noted

<sup>9</sup> BENEDICT: *Loc. cit.*

<sup>10</sup> MARTIN: This journal, 1905-1906, xv, p. 316.

that these changes in irritability may be produced by conditions or agencies which are not able to produce direct contractions. Certain facts, such as the one above cited, appear to indicate that heart muscle may undergo changes in irritability which are somewhat analogous to the changes in irritability of skeletal muscle, and that such changes are indicated by tonus changes and can be produced by agents which are in themselves unable to cause a series of beats.<sup>11</sup> We are indeed justified in regarding the tonus changes as in the nature of effects, but such a view does not imply that agents which produced the tonus changes have in no wise altered the capability of the strip to give a rhythmic series of beats. On the contrary, it is only reasonable to believe that a strip cannot be so affected as to alter distinctly its tonus without at the same time altering its susceptibility to stimuli. There is no apparent justification for the assumption commonly made by writers on heart beat that the only effects of importance which substances may have upon heart tissue is direct or immediate stimulation to rhythmic activity. The barest facts of the subject show such a position to be untenable. How, upon such an assumption, are we to explain the fact that a fresh strip immersed in serum or Ringer's solution will remain practically quiescent until decay takes place, whereas it will beat for many hours in either of these solutions if previously left in sodium chloride solution for an hour or two? As long as the assumption is made that substances act upon heart tissue only by direct stimulation to rhythmic activity, any explanation of these facts based upon the diffusion of salts will not be satisfactory, since it should be assumed that in the first hour or two of subsequent immersion in Ringer's solution or in serum the ratio of ions in the strip becomes practically what it was in the fresh strip. The suggestion that some inhibitory combination of ion with protein exists in the tissue, and that this combination can be broken down only slowly, is not a satisfactory explanation, in view of the fact above stated regarding results of brief immersion in oxalate, followed by treatment with Ringer's solution. Attempted explanations based upon the oxygen supply are no more satisfactory.

The series of tonus changes occurring in a heart strip appears to the writer as very significant. Upon immersion in sodium

<sup>11</sup> It is not implied here that increase in tonus necessarily means increase in irritability, except within certain limits.



chloride solution the fresh strip loses tonus continuously. During a certain period of the immersion the strip gives a series of beats which begin almost at a maximum in rate and amplitude and then gradually decline. Simultaneously with the falling off of the tonus the beats become more and more feeble, until the series ceases with the strip in a relaxed condition. Subsequent immersion in Ringer's solution is followed by a slight but distinct increase in tonus, accompanied by a renewal of the rhythmic series, which will then continue for several hours. The increased tonus is maintained in Ringer's solution for some time but is not augmented. Ringer's solution is the best fluid we have for maintaining heart strips in a fixed state of tonus, and is also the best solution we possess for maintaining a long series of beats in heart tissue.

It is evident, by the decreasing amplitude of the sodium chloride series of beats, that the tissue is either losing the stimulating agent or that it is becoming less irritable to this stimulating agent. Now, if the only factor involved were loss of the stimulating agent, the fresh strip should beat in Ringer's solution, since it beats there for hours subsequent to the immersion in sodium chloride solution. As further evidence that the strip has lost something besides the stimulating agent during the sodium chloride series, we have the fact that at the end of this series the tissue is practically unresponsive to mechanical or electrical stimulation. There remains, then, the obvious conclusion that during the immersion in sodium chloride solution the tissue has lost in irritability through loss of too much of the tonus-increasing substance. This point of view is further indicated by the fact that all of the substances (seven or more in number) which have so far been proposed as agents for renewal of beats after the sodium chloride arrest cause a more or less marked increase of tonus when applied to heart tissue. Further facts bearing upon this point will be discussed below.

The removal of calcium by oxalate and subsequent immersion in Ringer's solution is, then, one method by which heart strips can be made to give a series of contractions without previous treatment with pure sodium solutions. Martin has recently suggested another, which consists in moistening the strip with calcium chloride solution while hanging in a moist chamber through which air and carbon dioxide are successively passed. After several repetitions of this process the strip begins a series of beats which may last for some time. Martin's conclusions from this result will be con-

sidered later. The number of factors involved is so great as to make this experiment difficult to interpret. Martin states that the carbon dioxide both decreases tonus and furnishes a direct stimulus to rhythmic activity. The latter part of this statement lacks experimental demonstration, since wherever beats have followed the use of carbon dioxide there was present either a large amount of sodium chloride solution or of calcium chloride solution, making it possible that the carbon dioxide effect may have been indirect in each case. The separate action of carbon dioxide and of oxygen as reported by Martin will be discussed later.

A method which the writer has recently found for the initiation of beats in a fresh heart strip is to immerse the tissue in a solution of galactose<sup>12</sup> approximately isotonic with 0.7 per cent sodium chloride solution. Treatment of a strip with such a solution is followed by a series of powerful beats which begins within five minutes after immersion and lasts from three quarters of an hour to an hour and a half. The usual duration of the series is slightly under an hour. The beats in galactose solution are very powerful and rapid at first, accompanied by a marked inability of the strip to relax completely. The relaxation may become less after each contraction, until finally the base line becomes level with the top of the original contractions and the beats cease. Towards the end of the series the beats tend to group themselves somewhat. A series quite analogous to this but of much shorter duration is sometimes obtained after immersion of a fresh strip in calcium bromide solution.<sup>13</sup> The significance of the result with galactose solution will be discussed further in connection with the questions of the rôle of non-electrolytes and the origin of the inner stimulus.

**The latent period.** — It is seldom that a fresh heart strip begins to beat at once upon immersion in sodium chloride solution. The series of beats is usually preceded by a "latent period," during which time the strip lies perfectly quiet. This latent period lasts usually from half an hour to an hour and a half. It has been given numerous interpretations. Many of these explanations have failed to take into account the fact that after exhaustion in sodium chloride solution the strip will beat for hours in a Ringer's solution, although it will not do so before such treatment. Thus

<sup>12</sup> A  $n/4$  solution of KAHLBAUM's galactose was used. This solution gave no test for calcium with oxalate, and yielded only a faint flame test for sodium.

<sup>13</sup> BENEDICT: This journal, 1905, xiii, p. 192.

Howell<sup>14</sup> suggested that potassium ions are *per se* inhibitory to heart tissue, and that the latent period represents the time required for the outward diffusion of these ions. When we remember that after cessation of beats in a sodium chloride solution the strip will beat for hours in a medium containing potassium ions (Ringer's solution), while it will not beat at all in this solution without some special previous treatment, Howell's explanation appears inadequate. Recently Martin<sup>15</sup> has laid emphasis upon the relation of oxygen to heart activity, and puts forward another hypothesis, or rather a combination of hypotheses, to explain the sodium chloride latent period. In regard to this point Martin says: "It is assumed that spontaneous rhythmicity in ventricular tissue depends upon two factors: (1) the presence within it of certain definite products of its own metabolic activity, one of which may be carbon dioxide, these constituting the 'inner stimulus' of Langendorff; (2) the presence of diffusible calcium ions whose chief function is to promote those reactions between the tissue and oxygen which are essential to its activity. The normal non-automacity of the ventricle is explained in part by the author's former assumption that its calcium content is in such a combination with the tissue substance that it is not diffusible and so is not active except when, as the result of the application of an external stimulus, enough of it is changed from the inactive to a diffusible active combination for the requirements of that particular response, and in part also by the supposition that the metabolic activity of the tissue, which depends upon the presence of diffusible calcium ions, is normally too restricted to produce substances which will suffice as stimuli to automatic contractions."

A consideration of a few primary facts of heart-beat phenomena will demonstrate that this hypothesis of Martin's cannot be accepted. A fresh strip will not beat in Ringer's solution, or in serum, where calcium ions are present in abundance. Removal of calcium ions from a strip by means of oxalate solution is followed by an immediate series of beats in Ringer's solution. If the treatment with oxalate solution be very brief and followed by several rinsings in sodium chloride solution, the latent period in this latter solution may be very frequently abolished, and a typical sodium chloride series gotten under way at once. The main

<sup>14</sup> HOWELL: This journal, 1901-1902, vi, p. 206.

<sup>15</sup> MARTIN: This journal, 1906, xvi, p. 202.

assumption in Martin's hypothesis, namely, that the latent period is largely due to a lack of diffusible calcium combinations, cannot well be reconciled with these results. Furthermore, Martin assumes that the latent period is due to deficient oxidation, yet he points out that an excess of oxygen prolongs the latent period, and that immersion of the strip in a solution of sodium chloride from which the oxygen has been previously removed by boiling is followed by a series of beats after an exceedingly short latent period.

Oxygen gas, furnished after the sodium chloride exhaustion, will produce beats at once, while it has an exactly opposite effect before the sodium chloride series begins. There are several other substances which act in a similar manner. Sodium carbonate cannot produce beats in a fresh strip, but can produce and maintain them for long periods of time after the sodium chloride exhaustion. Ringer's solution, dextrose, and lithium chloride are further examples of substances which cannot produce beats in a fresh strip, but can do so after the sodium chloride series. The fact that the same substances may have diametrically different effects upon heart tissue at different times indicates that the tissue undergoes changes in irritability. Upon the hypothesis put forward by the writer earlier in this paper we may readily explain the action of oxygen gas in inhibiting the initiation of beats at one time and in renewing and maintaining them at another. Oxygen gas tends to increase tonus in heart tissue, and therefore prevents as rapid loss of tonus in heart strips in sodium chloride solution as takes place in the absence of oxygen. Hence the *prolongation of the entire series* of sodium chloride phenomena by means of oxygen gas. The same explanation applies to the other substances which behave like oxygen in this respect. Upon this basis we should expect that carbon dioxide, a substance which causes a marked loss of tonus in heart strips, would cause a marked shortening in the sodium chloride series of phenomena. This is exactly what takes place. Immersion of a strip in sodium chloride solution saturated with carbon dioxide causes, according to Martin (who gave the fact an entirely different interpretation, which will be considered later), a marked fall in tonus and a practically complete abolition of the latent period, followed by a very short series of feeble beats.

In view of these facts the most rational interpretation of the sodium chloride latent period appears at present to be that it

represents the time required by the strip to reach an optimum degree of tonus for rhythmic activity.

#### THE ORIGIN OF THE INNER STIMULUS.

The production of an immediate series of beats by carbon dioxide in conjunction with sodium chloride solution was interpreted by Martin as evidence in favor of Langendorff's hypothesis that the products of the heart tissue's own metabolic activity are the stimuli to rhythmic contractions; and as a result of this, and interesting observations of the action of oxygen gas and calcium upon heart strips, he has taken the position that the balance of evidence is at present in favor of Langendorff's hypothesis as opposed to the position commonly taken by writers on the subject in this country, namely, that the inorganic constituents of the blood furnish the stimulus to rhythmic activity.

Martin's experimental evidence in favor of Langendorff's hypothesis is very meagre, namely: (1) Carbon dioxide, which is probably a product of heart muscle's activity, produces an immediate series of beats in a fresh strip immersed in sodium chloride solution; (2) Moistening a strip with calcium chloride solution and hanging it in a moist chamber through which air, or oxygen and carbon dioxide are successively passed is followed by a series of beats, and (3) Oxygen gas will cause a renewal of beats after exhaustion in sodium chloride and will prolong the series in sodium chloride or Ringer's solution.

The fact that oxygen gas can renew beats after the sodium chloride series is no evidence in favor of Langendorff's hypothesis. Calcium chloride, cane sugar, lithium chloride, and sodium carbonate all have the same effect to varying degrees. Furthermore, oxygen, which certainly should not retard oxidative metabolism in the tissue, prolongs the sodium chloride latent period.

The only fact presented by Martin which might be interpreted as somewhat favoring Langendorff's hypothesis is his result with carbon dioxide. Used in large quantities, this gas produces beats at once in a fresh strip immersed in sodium chloride solution. Since it is probably a product of heart-muscle metabolism, Martin concludes that the balance of evidence favors Langendorff's hypothesis. That this inference is entirely unwarranted is shown by the fact that beats can readily be produced in fresh strips by

substances which are not products of heart tissue's activity. Treatment of a fresh heart strip with oxalate, followed by Ringer's solution, produces beats at once. Galactose, also not a product of heart-tissue metabolism, gives rise to an immediate series of beats in a fresh strip. It appears that Martin was premature in his acceptance of Langendorff's hypothesis, and that the sum of present evidence speaks strongly against its acceptance.

#### THE SODIUM CHLORIDE SERIES AND THE SODIUM CHLORIDE ARREST.

The series of beats in sodium chloride solution usually begins almost at a maximum in rate and amplitude. This is very soon followed by a gradual and regular decrease in the amplitude of the beats until the zero point is reached. The series is accompanied by a loss of tonus in the strip and usually lasts from one to three hours.

In a previous paper<sup>16</sup> it was suggested that the sodium chloride arrest is due to too great loss of tonus (irritability) in the strip, this loss of irritability being due, in part at least, to excessive diffusion of calcium ions from the tissue. This explanation was based upon the facts that the decrease in amplitude of the beats is accompanied by loss of tonus, and the further observation that all the substances which will cause renewal of beats after the sodium chloride exhaustion act to increase tonus in heart tissue.

Lingle<sup>17</sup> reported in 1902 that a heart strip will renew its activity after sodium chloride exhaustion upon suspension in oxygen gas or air, or upon addition of hydrogen peroxide to the solution in which exhaustion had taken place. Martin<sup>18</sup> has recently studied the effect of oxygen gas upon heart strips at some length, and concludes, as did Lingle, that the cessation of beats in sodium chloride is due to a lack of available oxygen. Martin based his conclusions chiefly upon the following facts: (1) Oxygen gas will cause renewal of beats after sodium chloride exhaustion, and will delay the onset of such exhaustion if passed through the solution in which the sodium chloride exhaustion is taking place. (2) Calcium chloride cannot cause renewal in the total absence of oxygen,

<sup>16</sup> BENEDICT: This journal, 1905, xiii, p. 192.

<sup>17</sup> LINGLE: This journal, 1902, viii, p. 83.

<sup>18</sup> MARTIN: This journal, 1905-1906, xv, p. 303.

that is, in solutions artificially completely deoxygenated. (3) Addition of oxygen to solutions may, under certain circumstances (as in a series of beats in Ringer's solution), cause an improvement in the series.

In the interpretation of his results with oxygen gas Martin has proceeded upon the assumption that the oxygen effect is due entirely to the increased amount of this substance available for oxidative purposes. Such an assumption is unwarranted and may readily lead to error in interpretation of results. The nature of the normal oxygen supply of heart-muscle cells is in no way similar to the oxygen treatment which Martin employed. It appears very probable that a large excess of gaseous oxygen, such as is supplied by bubbling the gas continually upon or near the tissue, or suspending the strip in an atmosphere of oxygen, would have a very decided effect aside from supplying such oxygen as is needed for oxidative purposes. The fact that heart tissue does utilize some oxygen is no evidence that excessive amounts of this substance have no effect outside of offering oxygen for metabolic activity. Dextrose, a substance which muscle cells very probably utilize, can cause a brief but decided renewal after the sodium chloride arrest, but we may not conclude from this fact that the arrest is due to a lack of nutrient material and that the strip utilizes dextrose as a source of energy. There is, then, no justification in the assumption that an enormous excess of gaseous oxygen acts only, or indeed at all, through supplying such oxygen as is needed for favorable oxidation. The fact that an excess of oxygen retards the development of beats in a fresh strip immersed in sodium chloride solution is conclusive evidence that large amounts of this substance have an effect aside from increasing favorable oxidation. Correctly interpreted, it would appear that Martin's work in this connection represents a study of the effects of excessive amounts of oxygen upon heart strips, and that his results have in no way called into question the validity of Howell's statement that aqueous solutions of inorganic salts contain a sufficient supply of oxygen for heart tissue, so that this factor may be disregarded in ordinary work.

Martin further concludes that because calcium cannot, in the *complete* absence of oxygen, cause renewal of beats after the sodium chloride arrest, the calcium effect in a normal supply of oxygen is therefore due to the property of calcium ions to render the oxygen

present more available for the tissue. It is difficult to find any justification for this hypothesis. There is no reason to assume that if calcium acts otherwise than by promoting oxidation within the tissue, it should be able to incite the tissue to rhythmic activity in the *complete absence* of an oxygen supply, as is the apparent basis of Martin's assumption. It would be only rational to suppose that the tissue must have some external oxygen available if it is to exhibit the calcium effect. Furthermore, if it is to be claimed that calcium acts by promoting favorable oxidation, must not the same rôle be ascribed to lithium chloride, dextrose,<sup>19</sup> and cane sugar, all of which substances cause brief but decided renewal after the sodium chloride arrest? There appears, then, to be no evidence that calcium ions act upon heart tissue by promoting oxidation.

Oxygen, calcium chloride, dextrose, cane sugar, and sodium carbonate all act to increase tonus in heart strips, and all cause renewal after the sodium chloride exhaustion. The three strongest tonus-increasers (calcium chloride, sodium carbonate, and oxygen) cause instantaneous and prolonged renewal. The most rational explanation of the common ability of these substances to cause a renewal of beats after the sodium chloride exhaustion appears to be found in the property of increasing tonus in heart tissue which is common to all of them.

The sum of present evidence appears to favor the view that the sodium chloride exhaustion is due to excessive loss of tonus in the tissue. There is no basis for the assumption that the arrest under the usual conditions is in any sense a phenomenon of asphyxiation.

THE RENEWAL AND MAINTENANCE OF BEATS AFTER THE SODIUM CHLORIDE ARREST, INCLUDING SOME OBSERVATIONS ON THE RELATIVE RÔLES OF ANIONS AND CATIONS, AND ON THE RELATION OF NON-ELECTROLYTES TO VENTRICULAR TISSUE.

The agents which will cause renewal of beats after exhaustion of a strip in sodium chloride solution are: (1) calcium chloride,

<sup>19</sup> PACKARD, in an article attempting to support MATHEWS' theory of respiration (This journal, 1907, xviii, p. 164), has cited MARTIN'S results with heart strips and oxygen, and offers the renewal of beats by dextrose after the sodium chloride arrest as further evidence in favor of MATHEWS' theory. He has not noted that lithium chloride, which is certainly not a depolarizer, is as good a renewing agent as is dextrose, while calcium chloride and sodium carbonate are many times as effective in this respect as is dextrose or cane sugar.



(2) dextrose, (3) lithium chloride, (4) cane sugar, (5) oxygen gas, air, and hydrogen peroxide, (6) sodium carbonate, (7) Ringer's solution. It is very interesting to note that not one of these substances is capable of producing beats in a fresh strip. It is of particular interest that sodium carbonate is unable to occasion beats in the fresh tissue, since other compounds of sodium are the only electrolytes which produce beats without previous treatment with some other agent. It was this fact, presented by the writer in a previous paper,<sup>20</sup> which led to the suggestion that it is the anion which stimulates heart tissue directly, while the chief function of the cation is to maintain the tissue in a degree of tonus best suited to activity. The writer has not yet investigated further the relative rôles of anion and cation in relation to heart activity, but it appears very probable that the anion has a more positive effect than is usually ascribed to it. There have been several facts mentioned in the literature which would have been adequately explained by assuming that the anion plays an active part, wherein the conclusions have been distorted simply because of a failure to recognize that the anion may have a positive rôle. Miss Moore<sup>21</sup> found that sodium sulphate would renew beats in the lymph hearts of the frog after exhaustion in sodium chloride solution. Upon the basis of this result she criticises Howell's conclusion that calcium is necessary in heart beat because the "sodium sulphate precipitates calcium and yet is able to cause renewal. . . ." This criticism is unfounded, inasmuch as there is no reason to believe that sulphate could precipitate calcium in the concentration in which this substance exists in the serum or tissue. The result which Miss Moore gives indicates that the anion exerts a positive action in the experiment cited. Garrey<sup>22</sup> reported that the oxalate, phosphate, carbonate, citrate, or fluoride of sodium will cause more powerful twitchings in fresh skeletal muscle than does sodium chloride. Garrey appeared to regard all of these salts as calcium precipitants or "inactivators," of which the sulphate certainly is not one, nor is it probable that the citrate would have any such effect in the practically neutral fluids of the organism. These effects would better be ascribed to the different anions employed, and offer further corroboration of the view that this ion has a positive function in relation to certain tissues.

<sup>20</sup> BENEDICT: *Loc. cit.*

<sup>21</sup> MOORE: This journal, 1902, vii, p. 315.

<sup>22</sup> GARREY: This journal, 1905, xiii, p. 186.

In considering the renewal of beats produced by cane sugar and dextrose after the sodium chloride exhaustion, the interesting question arises whether non-electrolytes may play a positive rôle in the initiation or maintenance of beats in heart tissue. Howell assumed that the non-electrolytes are entirely passive in regard to heart tissue, producing their effects merely by permitting outward diffusion of ions from the tissue. Lingle also has maintained that non-electrolytes can have no positive effect in the production of beats in heart tissue. Such an assumption is made with very little, if any, evidence to justify it. In a previous paper<sup>23</sup> the writer suggested that non-electrolytes do, under certain conditions, have a positive effect upon heart tissue, inducing changes in irritability, as is indicated by the tonus changes which occur after immersion of a heart strip in solutions of certain non-electrolytes. Since the publication of that paper Carlson<sup>24</sup> and Eggers<sup>25</sup> have both brought forward further evidence that the action of non-electrolytes upon heart and nerve tissue is not necessarily negative. Results obtained recently by the present writer appear to demonstrate conclusively that certain non-electrolytes can have a positive effect in initiating rhythmic contractions in ventricular tissue. Lactose solution, isotonic with 0.7 per cent sodium chloride solution, will not cause renewal of beats after the sodium chloride arrest. Inasmuch as dextrose will cause such renewal, either before or after immersion of the strip in lactose solution, we may infer that the lactose solution is not toxic and that dextrose exerts a positive action which lactose does not exert. Finally, as was stated above, immersion of a fresh heart strip in galactose solution, isotonic with 0.7 per cent solution of sodium chloride, is followed at once by a rhythmic series of beats. Such treatment of a strip is a sure and rapid means for obtaining a series of powerful contractions in a fresh strip, surpassing in this respect any electrolyte or combination of electrolytes so far studied. It is the intention of the writer to investigate further the action of galactose solutions in relation to heart and skeletal muscle.

#### CONCLUSIONS.

I. Certain substances affect ventricular tissue so as to alter its irritability or power of responding to stimuli, without necessarily

<sup>23</sup> BENEDICT: This journal, 1905, xiii, p. 192.

<sup>24</sup> CARLSON: This journal, 1906, xvi, p. 221.

<sup>25</sup> EGGERS: This journal, 1907, xviii, p. 64.

inciting rhythmic contractions. This fact permits of a reinterpretation of many heart-tissue phenomena, and brings heart muscle into line with other muscular tissue in a way not commonly recognized.

2. The sodium chloride latent period represents the time required by the strip to reach a condition of tonus suited to rhythmic activity. It is not caused by lack of calcium ions or by a lack of available oxygen, as has been suggested by Martin.

3. Under the ordinary conditions the sodium chloride arrest is due to loss of irritability rather than to loss of the stimulating agent. Under the usual circumstances the arrest cannot be regarded as in any sense a phenomenon of asphyxiation, as has been suggested by some writers.

4. The balance of experimental evidence is at present strongly against the acceptance of Langendorff's hypothesis that the products of the heart tissue's own metabolic activity constitute the stimuli to rhythmic contractions.

5. Martin's conclusion that an excess of oxygen and diffusible calcium compounds both act by increasing favorable oxidation cannot be accepted. It is true that both of these substances may act indirectly upon heart tissue, but there is no evidence that either one directly increases favorable oxidation. The facts point against such an assumption.

6. Under certain conditions non-electrolytes may induce a series of beats in ventricular tissue.

7. The anion probably plays an active rôle in the action of salt solutions upon heart tissue.

8. A method is presented for obtaining a series of beats in a fresh ventricular strip without the use of any salt solutions.

It is a pleasure to express my indebtedness to Professor Michael F. Guyer of the University of Cincinnati and to Professor Lafayette B. Mendel of Yale University for suggestions and criticisms regarding this work.

## THE EXCRETION OF BROMIDES BY THE KIDNEY.

BY WORTH HALE AND CASRIEL FISHMAN.

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THE use of the bromides in medicine is of so much importance on account of their wide use as depressants of the central nervous system that the investigation of their relation to the physiological functions of the various organs has always been of much interest. In consequence, the literature of the subject has become very extensive, and numerous reports have been made on the excretion of these salts in the urine, the early reports, however, often being quite at variance with those which have appeared recently. The recent reports, on the other hand, while not always agreeing in detail, have supplemented these early reports, and have added to our knowledge of the elimination of the bromides much not only of scientific but also of therapeutic value.

Bowditch (1), in 1868, investigated the excretion of potassium bromide in the urine, experimenting with a number of students, each receiving 20 grains of this salt. The urine was collected at the following intervals after administration: ten minutes, thirty minutes, one, two, four, six, eight, thirteen, eighteen, twenty-five, thirty-two, and forty-two hours. The earliest period at which the presence of bromides was shown distinctly was thirty minutes, the latest thirty-two hours. In a second series of experiments traces were found as late as fifty-two hours following a dose of 10 grains. To determine the amount excreted by the kidney five doses were given at intervals of six hours and the urine collected for thirty-two hours, the period ending eight hours after the last dose. A quantitative examination gave 28.72 grains, or 57.44 per cent of the amount taken.

Bowditch, quoting Namias (2), also pointed out that the repeated administration of bromides delayed their elimination greatly, an

epileptic showing traces in the urine as late as the fourteenth day following the withdrawal of the drug.

Nencki and Schoumow-Simankowsky, (3) working with dogs, were able to show that the excretion of bromides was much more markedly delayed, however, for after a period of nearly four months they found traces in the urine of a dog which had been given 53 gm. of sodium bromide in divided doses. In this same series of experiments it was also shown that the bromides could replace the chlorides in the body—notably in the gastric juice—occurring there as hydrobromic acid.

It is of interest to note here, however, that this close relationship between the chlorides and the bromides had been demonstrated by Bill (4) nearly forty years previously. He proved, in a number of experiments on men, that the introduction of bromides into the body always greatly increased the chloride content of the urine, and from this he concludes that the bromide of sodium is a physiological substitute for sodium chloride, taking its place in the tissues, and that it may remain in the body for some time, or in fact until replaced by the sodium chloride ingested with the food. In support of this it was found that bromides could be detected in the urine ten and even fourteen days after the drug had been withdrawn.

The results obtained by Hondo (5) from a series of experiments on patients differ to some extent. He reports that the bromides, if given in several doses, are excreted for the most part during the first ten days, although some still remains in the tissues, and traces were found by him as late as the fortieth day. If, however, the sodium chloride ingested in the food be reduced in amount, the elimination of bromides proceeds much more slowly, this again supporting Bill's earlier observation.

Von Wyss (6), the latest worker on this subject, showed by his experiments that the excretion of bromides falls behind the ingestion of these salts to a very marked degree. In one experiment in which 7.763 gm. of bromine, taken as sodium bromide, had been given, the total excretion for a period of ten days was only 2.384 gm. calculated as bromine. He found, however, that a balance was established after a time between the amount taken and that excreted, this balance appearing about the seventeenth day, but the amount excreted never became so great as the amount taken. This indicated a large excretion along other channels or a storing

up of bromides in the body; and the latter was shown to be the case, for large amounts were isolated from the brain and blood especially, but also from various other organs in lesser amounts. Von Wyss was also able to prove from the positive side the antagonism between chlorides and bromides suggested by Hondo and Bill, for increasing the amount of sodium chloride ingested hastened the elimination of bromides very considerably.

It will be observed, from this brief survey of the literature, that, with the exception of Bowditch, all experiments have been made upon subjects who had been given bromides for some time. We decided, therefore, that it might be worth while to supplement Bowditch's work, using the newer methods for quantitatively estimating the bromides of the urine, since it did not seem probable that such great disparity should exist between the elimination of a single and successive doses, either to the degree given by Namias or more especially by Nencki and Hondo. Anten (7), in working with the closely related iodides, was able to demonstrate, nevertheless, that the excretion of single doses of this salt was very rapid while the exhibition of the same amount in divided doses increased markedly the time necessary for complete elimination, — a fact which suggested that the same might also be true for the bromides. In our experiments we have attempted to point out how far this analogy holds and to what degree the results of Bowditch are dependable.

The difficulty of determining small amounts of bromides in the presence of large amounts of chlorides and organic material as in the urine, made it necessary to determine the relative value of several of the methods used in the quantitative estimation of bromides. Colorimetric methods, although applicable, are not direct enough to be accurate, especially for the small amounts which we expect to determine in our experiments. The silver method of Buchner was also found to be too inaccurate for the small amounts to be determined. A method slightly altered from that given by Classen (8) for estimating bromides in water solution with large amounts of chlorides, proved to be well suited to our needs. This method had also been found by Von Wyss (6) to give good results.

This method is based on the fact that when a mixture of the bromides and chlorides is boiled with potassium bichromate in a

solution of sulphuric acid, free bromine is liberated before the chlorides become affected by oxidation.

100 c.c. of the urine, with 15 c.c. of a 33 per cent solution of sodium hydrate, is evaporated to complete dryness in a porcelain evaporating-dish, either on a water or a sand bath. The dried total solids are then ignited at a low heat, gradually elevating the temperature until the mass is completely carbonized. This is then removed, when cool, to an agate mortar, and after being pulverized is again returned to the evaporating-dish or porcelain crucible and ignited at a red heat to a dark gray ash. This contains all of the non-volatile salts of the urine, including the bromides together with an excess of alkali. The ash is now heated with water and washed through a filter into a Florence flask of about 250 c.c. capacity to remove all soluble matter. To this is added slowly a sufficient quantity of dilute sulphuric acid to make the solution acid, and afterwards to this are added 20 c.c. of a 50 per cent sulphuric acid and 10 gm. of potassium bichromate, dissolved in a small amount of water. The solution and washings will make a volume of about 175 or 200 c.c. This flask is now connected with a smaller flask, containing a neutral or at most a very slightly acid solution of potassium iodide (1 gm. in 10-15 c.c. of water) which is placed in a jar of running water to aid in the condensation of the bromine as it is vaporized and passed into the iodide solution. The flask containing the bromides and chlorides is boiled until a volume of about 50 c.c. remains and the distillate, consisting of bromine and water vapor, being condensed in the potassium iodide solution, liberates free iodine with the formation of potassium bromide. If the distillation is carried on to the point mentioned above, little bromide remains, but to make certain steam may be passed into the flask containing the combined salts for about fifteen minutes to break up any traces that may remain, although we have not found this last step necessary. The iodine set free is then titrated against  $n/20$  or  $n/40$  sodium thiosulphate, using starch solution as an indicator. From the amount of iodine obtained in this way we may estimate the amount of bromine liberated and the total sodium bromide in the original sample, the following values being used:

1 c.c.  $n/20$  sodium thiosulphate equals 0.0040 bromine.

1 c.c.  $n/20$  sodium thiosulphate equals 0.0052 sodium bromide.

In trial experiments using known quantities of sodium bromide, the following results were obtained:

1. 25 mgm. sodium bromide, in 100 c.c. urine, gave 24 mgm.
2. 10 mgm. sodium bromide, in 100 c.c. urine, gave 8.5 mgm.
3. 10 mgm. sodium bromide, in 100 c.c. urine, gave 9.36 mgm.

Having thus satisfied ourselves as to the accuracy of the method, the following experiment was carried out:

#### EXPERIMENT I.

Subject, adult male; weight, 135 pounds; occupation, student. Given 1 gm. of sodium bromide by the stomach, April 28, 1906, 8 A. M. Mixed diet.

Time.	Hours urine.	Amount of urine.	Specific gravity.	Sodium bromide excreted.
<b>April 28</b>		<b>c.c.</b>		<b>gm.</b>
10 A. M.	2	80	1.031	0.0041
12 M.	2	69	1.030	0.0062
2 P. M.	2	60	1.035	0.0041
5 P. M.	3	117	1.023	0.0032
6 P. M.	1	107	1.015	0.0030
8 P. M.	2	103	1.030	0.0026
10 P. M.	2	90	1.030	0.0031
<b>April 29</b>				
8 A. M.	10	265	1.032	0.0054
11 A. M.	3	79	1.028	0.0052
2 P. M.	3	120	1.030	0.0135
5 P. M.	3	160	1.027	0.0082
7 P. M.	2	73	1.027	0.0052
11 P. M.	3	150	1.032	0.0093
<b>April 30</b>				
7 A. M.	8	160	1.034	0.0098

The total amount of sodium bromide excreted during the forty-eight hours of this experiment amounts to 0.0829 gm., or only 8.29 per cent of the amount taken. It is quite obvious, therefore, that the collection and examination of the urine for the excretion of bromides should be made for a considerably longer period. This result is in contrast to the findings of Bowditch, and is more in accord with the results of the later investigators. The experiment was accordingly repeated, tests being made with day and night intervals instead of the more frequent intervals of the earlier experiment. The following table gives our results:



EXPERIMENT II.

Subject, adult male; weight, 136 pounds; occupation, student. Given 2 gm. of sodium bromide by stomach, May 15, 1906, 8 A. M.

Date.	Hours urine.	Amount of urine.	Specific gravity.	Sodium bromide per 100 c.c.	Total sodium bromide.
May 15	12	520	1.020	0.0114	0.0594
" 15-16	"	410	1.022	0.01196	0.0490
" 16	"	610	1.021	0.01040	0.0634
" 16-17	"	540	1.022	0.00832	0.0449
" 17	"	640	1.023	0.01456	0.0931
" 17-18	"	380	1.032	0.01976	0.0750
" 18	"	325	1.033	0.01248	0.0405
" 18-19	"	470	1.031	0.00884	0.0415
" 19	"	305	1.031	0.01456	0.0440
" 19-20	"	210 <sup>1</sup>	1.034	0.01248	0.0262
" 20	"	290	1.030	0.00900	0.0261
" 20-21	"	215	1.033	0.00416	0.0089
" 21	"	300	1.030	0.00208	0.0062
" 21-22	"	295	1.029	0.01248	0.0374
" 23	"	560	1.028	0.00728	0.0407
" 25	"	600	1.021	0.00416	0.0249
" 25-26	"	460	1.022	0.00572	0.0263
" 26	"	1300	1.018	0.00468	0.0608
" 26-27	"	700	1.018	0.00520	0.0364
" 29	"	790	1.022	0.00416	0.0328
" 30	"	900	1.017	0.00520	0.0468
" 30-31	"	625	1.022	0.00424	0.0265
<sup>1</sup> Subject suffering from an attack of tonsillitis.					

As Experiment II was interrupted, and as the urine still showed a large daily excretion (0.026 gm. on the sixteenth day), it became necessary to do a third experiment. In this one it was

deemed unnecessary to make observations at such frequent intervals, since the excretion evidently proceeded very slowly. Accordingly daily observations were made only during the first week, and thereafter twice a week, until the test for bromides showed mere traces of the drug in the daily excretion. The following table gives the results of this experiment in detail:

## EXPERIMENT III.

Subject, adult male; weight, 150 pounds; occupation, student. Given 2 gm. of sodium bromide by the stomach, September 28, 1907, 9 P. M.

Day.	Date.	Hours urine.	Specific gravity.	Amount urine.	Sodium bromide per 100 c.c.	Total sodium bromide.
1	Sept. 29	12	1.022	c.c. 425	gm. 0.0095	gm. 0.0403
2	" 30	24	1.020	1275	0.0067	0.0854
3	Oct. 1	"	1.020	1150	0.0088	0.1010
4	" 2	"	1.017	1100	0.00572	0.0629
5	" 3	"	1.024	1230	0.00572	0.0703
6	" 4	"	1.022	1300	0.00468	0.0608
7	" 5	"	1.025	1380	0.00884	0.1219
10	" 8	"	1.022	1220	0.00442	0.0539
14	" 12	"	1.025	1420	0.00416	0.0590
17	" 15	"	1.024	1280	0.00260	0.0332
21	" 19	"	1.020	1530	0.00312	0.0477
28	" 26	"	1.020	1370	0.00052	0.0070
31	" 29	"	1.024	1180	0.00104	0.0122
35	Nov. 2	"	1.026	1130	0.00130	0.0146
38	" 5	"	1.024	1070	0.00052	0.0055
42	" 9	"	1.024	875	0.00078	0.0068
45	" 12	"	1.028	800	0.00104	0.0083
49	" 16	"	1.010	1380	0.00026	0.0035
52	" 19	"	1.015	1250	0.00013	0.0016
56	" 23	"	1.024	1190	{ only a } { trace }	....

The results of this experiment agree in general with those of Experiment II, although the highest daily excretion appeared on the seventh rather than on the third day; but it will be noticed that the excretion on the third day in Experiment III was also very high per 100 c.c., the total being considerably less on account of the smaller diuresis. The following table shows a further remarkable regularity in the excretion, — more emphasized, too, by the difference in total diuresis in each case:

Experiment.	Period.	Total excretion in grams.	Total excretion in per cent.	Total diuresis.
II	<sup>days</sup> 6½	0.5778	28.9	<sup>c.c.</sup> 5235
III	6½	0.5426	27.1	7660

In Experiment I, during the first forty-eight hours, the total excretion was 8.29 per cent, following a dose of 1 gm.; in Experiment II during the same period 10.84 per cent of the 2 gm. taken appeared in the urine, figures which make it difficult to understand how Bowditch was able to find an elimination of 57 per cent in thirty-two hours. He used the potassium salt, however; and while it is possible that the different metallic ion has some influence on excretion, it is probable that after absorption all bromides are changed into the sodium salt in the blood, at least to a very large extent, and accordingly would be excreted very largely in such combination, comparing, therefore, very closely with the rate of elimination after a similar dose of sodium bromide.

In order to determine to what extent the metallic ion affected the rate of excretion, we decided to do another experiment, the regularity in the excretion of sodium bromide after single doses being so great, as has been shown above, suggesting that it would be a satisfactory method upon which a comparison of the rate of excretion of the various bromide salts might be based.

#### THE EXCRETION OF CALCIUM BROMIDE.

For this experiment we decided to use calcium bromide, maintaining as far as possible the conditions of the previous experiments. Our attention was especially called to this salt by a state-

ment made by Wood (9), in which he says that calcium bromide is absorbed and excreted very rapidly, attributing this to a report

#### EXPERIMENT IV.

Subject, adult male; weight, 155 pounds; occupation, student. Given 1.933 gm. of calcium bromide by the stomach, January 10, 1908.

Day.	Date.	Hours urine.	Specific gravity.	Amount urine.	Sodium bromide per 100 c.c.	Total sodium bromide.
				c.c.	gm.	gm.
1	Jan. 11	12	1.024	510	0.00884	0.0450
2	" 12	24	1.026	1100	0.00624	0.0686
3	" 13	"	1.014	1380	0.00611	0.0843
4	" 14	"	1.024	1130	0.0078	0.0881
5	" 15	"	1.024	1180	0.0091	0.1073
6	" 16	"	1.020	1160	0.00546	0.0633
7	" 17	"	1.018	1600	0.00598	0.0946
10	" 20	"	1.022	1220	0.00052	0.0063
14	" 24	"	1.024	1150	0.00494	0.05681
17	" 27	"	1.026	1170	0.00364	0.0425
21	" 31	"	1.021	1050	0.00384	0.0403
24	Feb. 3	"	1.020	1200	0.00286	0.0343
31	" 10	"	1.024	1000	0.00182	0.0182
35	" 14	"	1.023	1050	0.00130	0.0136
38	" 17	"	1.020	1220	0.00052	0.0063
42	" 21	"	1.025	1150	0.00052	0.0059
45	" 24	"	1.018	1350	0.00013	0.00175
49	" 28	"	1.022	1100	0.00013	0.00143
52	Mar. 2	"	1.027	980	{ mere trace / only }	....

made by See (10). A careful reading of this report fails to reveal any statement to this effect, however, the article dealing rather with comparisons between the halogen salts of calcium and other calcium preparations used in medicine.

To make our comparison of value it was necessary to give such

a dose of calcium bromide that the total bromine would equal the total bromine in 2 gm. of sodium bromide, the dose given in Experiments II and III, this amount of bromine being represented by 1.933 gm. of the calcium salt.

The subject of this experiment was the same as in Experiment III, the diet corresponding also in every respect with that of the former experiment. The table on page 40 gives our results in detail.

A surprising similarity in this experiment will be observed when compared with the results obtained in Experiments II and III.

Day.	Hours urine.	Experiment II. NaBr.	Experiment III. NaBr.	Experiment IV. CaBr <sub>2</sub> .
1	12	gm. 0.0594	gm. 0.0403	gm. 0.0450
2	24	0.1134	0.0854	0.0686
3	"	0.1381	0.1010	0.0843
4	"	0.1156	0.0629	0.0881
5	"	0.0856	0.0703	0.1073
6	"	0.0523	0.0608	0.0633
7	"	0.0151	0.1219	0.0946
Total sodium bromide . . .		0.5785 gm.	0.5426 gm.	0.5512 gm.
Total diuresis . . . . .		5235 c.c.	7660 c.c.	8060 c.c.

This is best shown by the above table, which gives the total excretion of bromides in each experiment, estimated as sodium bromide, for a period of six and a half days, giving in addition the total amount of urine secreted during this time.

Again, as in Experiments II and III, there appears no absolute relation between the total diuresis and the amount of bromide eliminated, although there is, no doubt, a relative one. Nor can it be stated definitely on what day the greatest elimination will take place. It may appear, as has already been noted, on the third, seventh, or, as in this experiment, on the fifth day; but this point is probably reached within the first few days, and after that the elimination sinks by degrees to zero, the time necessary for accomplishing this being less than sixty days after the ingestion of a single dose.

Approximating the total amount of bromide excreted by averaging the excretion for the periods when no examination of the urine was made reveals a further interesting similarity, the total amount of sodium bromide in Experiment III being about 1.462 gm.; in Experiment IV, 1.423 gm. This regularity is again demonstrated by comparing the time required for complete elimination, it being a trifle more delayed than with calcium in the case of sodium bromide. The results of the whole series of experiments agree so closely, however, that it seems an evident conclusion that the rate of excretion of bromides depends but slightly on the metallic ion.

#### CONCLUSIONS.

1. After a single dose the excretion of bromides is very much delayed, but probably to less extent than after a number of successive doses.
2. In spite of their close chemical relation, the bromides, after single doses, are excreted very much more slowly than the iodides.
3. The amount of diuresis does not seem to hold any absolute relation to the amount of bromide excreted.
4. The excretion of calcium bromide proceeds at about the same rate as sodium bromide.

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## THE EFFECT OF POTASSIUM IODIDE ON THE ACTIVITY OF PTYALIN.

BY C. H. NEILSON AND O. P. TERRY.

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and the Snodgrass Laboratory, City of St. Louis.]

### I.

THE clinical fact that potassium iodide has a disturbing influence on digestion led us to attempt these experiments. As it is well known that potassium iodide given by the stomach is absorbed and rapidly appears in the saliva, it occurred to us that possibly potassium iodide might modify starch digestion both in the mouth and in the stomach. The potassium iodide excreted in the saliva may cause a change in the amylolytic power of the saliva by increasing or decreasing the activity or the amount of the ptyalin.

Grützner,<sup>1</sup> Rübel,<sup>2</sup> Cole,<sup>3</sup> and Patten and Stiles<sup>4</sup> have determined the effect of potassium iodide and other neutral salts on the action of ptyalin. Grützner and Rübel state that potassium iodide in the lower concentrations decreases the amylolytic power of the ptyalin more than potassium chloride or bromide. Patten and Stiles found the same to be true for higher concentrations of potassium iodide. Patten and Stiles also state that it is a common observation that solutions of neutral salts in weak concentrations accelerate the action of ptyalin.

We find no references to work done upon the effect of potassium iodide given by the mouth upon the amylolytic power of the saliva.

<sup>1</sup> GRÜTZNER: Archiv für die gesammte Physiologie, 1902, xci, p. 195.

<sup>2</sup> RÜBEL: Archiv für die gesammte Physiologie, 1897, lxxvi, p. 276.

<sup>3</sup> COLE: Journal of physiology, 1904, xxx, p. 202.

<sup>4</sup> PATTEN and STILES: This journal, 1906, xvii, p. 26.

## II. THE EFFECT OF ADDING POTASSIUM IODIDE TO SALIVA IN THE TEST TUBE.

For these experiments the saliva was collected at the same time each day, — generally between 9.30 and 10 A. M. The mouth was rinsed with distilled water, and then a soft piece of paraffin was chewed. The first few centimetres of saliva were used and were collected in a graduate. The same amount was collected for each

TABLE I.

Contents of flask in cubic centimetres.	Amount of malt-ose produced.	Contents of flask in cubic centimetres.	Amount of malt-ose produced.
	mg.		
Starch paste . . . . . 75	} 140	Starch paste . . . . . 75	} 210
Potassium iodide 10 % . 15		Potassium iodide 10 % . 1	
Dilute saliva . . . . . 10		Distilled water . . . . . 14	
		Dilute saliva . . . . . 10	
Starch paste . . . . . 75	} 155	Starch paste . . . . . 75	} 212
Potassium iodide 10 % . 10		Potassium iodide 10 % . $\frac{1}{2}$	
Distilled water . . . . . 5		Distilled water . . . . . $14\frac{1}{2}$	
Dilute saliva . . . . . 10		Dilute saliva . . . . . 10	
Starch paste . . . . . 75	} 190	Starch paste . . . . . 75	} 210
Potassium iodide 10 % . 5		Potassium iodide 10 % . $\frac{1}{2}$	
Distilled water . . . . . 10		Distilled water . . . . . $14\frac{1}{2}$	
Dilute saliva . . . . . 10		Dilute saliva . . . . . 10	
Starch paste . . . . . 75	} 200	Control.	} 128
Potassium iodide 10 % . 3		Starch paste . . . . . 75	
Distilled water . . . . . 12		Distilled water . . . . . 15	
Dilute saliva . . . . . 10		Dilute saliva . . . . . 10	

experiment, — about 10 c.c. The diet was kept the same during the course of the experiments, for diet modifies the action of the ptyalin, as shown by observations which Dr. Neilson will soon report.

Two and one-half cubic centimetres of saliva were measured in a graduated pipette and then diluted to 100 c.c. with distilled water. In a 250 c.c. Erlenmeyer flask were placed 75 c.c. of a 2 per cent starch paste made of the best arrowroot starch. To this were added 15 c.c. of distilled water. Then the contents were warmed to 38.5° C. and 10 c.c. of diluted saliva added.

The flask was thoroughly shaken and placed in an incubator registering 38.5° C. for twenty minutes. At the end of this time



the contents of the flask were boiled. The decrease in volume, due to evaporation, was made up by adding distilled water. The amount of maltose produced by the action of the ptyalin was determined by Haine's method. This flask was the control and showed the power of the normal saliva.

To show the influence of potassium iodide, the volumes of the contents of the flasks were made the same as that of the control, — that is, 75 c.c. starch paste, 10 c.c. diluted saliva; but instead of adding 15 c.c. of water, a mixture of 10 per cent potassium iodide and water was added. Whatever the amount of potassium iodide, the mixture was always 15 c.c., so that the total volume was always 100 c.c.

The results of one set of experiments are shown in Table I. All the experiments show the same general result with occasional differences, but none such as to vitiate the results.

### III. THE EFFECT ON THE AMYLOLYTIC POWER OF THE SALIVA WHEN SECRETED WITH POTASSIUM IODIDE.

The method of procedure was different in this set of experiments. To test the effect of potassium iodide, patients were given potassium iodide by the mouth and the saliva collected, diluted, and its amylolytic power tested in the same manner as in Part I. For controls the saliva of a number of patients was tested for three days. The saliva was collected at the same time each day. The patients were kept on the same diet to insure no change in their saliva due to change of food.

The patients used for these experiments were acute surgical cases, such as broken bones, etc. At the end of the third day small doses of potassium iodide were given three times a day, and for each succeeding day, in most of the experiments, the amount given was gradually increased, until the patients complained of potassium iodide effects. Each day the amylolytic power of the saliva was determined as long as the potassium iodide was given. After an interval of three days the saliva was again tested. This served as a fourth control. The results are seen in Table II.

In Table I it is seen that potassium iodide has a marked accelerating action on the amylolytic power of saliva. The most marked influence is seen where the amount of potassium iodide added was one half of a cubic centimetre of 10 per cent solution.

TABLE II.

No.		Maltose.	Remarks.
1	Control. Average for three days .	mg. 17	Saliva collected 8.30 A. M., one and one-half hours after morning dose. Dilute saliva shows consider- able KI by the starch test.
	1st day, 150 gr. KI given . . .	40	
	2d day, 150 gr. KI given . . .	50	
	3d day, 150 gr. KI given . . .	65	
	4th day, 150 gr. KI given . . .	66	
	Potassium iodide stopped at end of 4th day		
2	Three days later . . . . .	22	No KI by starch test.
	Control. Average for three days .	50	Collection of saliva same as No. 1. Dilute saliva shows KI, but not so much as No. 1.
	1st day, 150 gr. KI given . . .	54	
	2d day, 150 gr. KI given . . .	62	
	3d day, 150 gr. KI given . . .	65	
	4th day, 150 gr. KI given . . .	62	
	Three days with no KI . . . . .	54	
3	Control. Average for three days .	108	Saliva collected at 9 A. M., two hours after last dose. No KI in dilute saliva by starch test.
	1st day, 45 gr. KI given . . . .	60	
	2d day, 45 gr. KI given . . . .	65	
	3d day, 45 gr. KI given . . . .	90	
	Three days with no Ki given . .	95	
4	Control. Average for three days .	20	Saliva collected same as No. 3. No KI in dilute saliva.
	1st day, 45 gr. KI given . . . .	16	
	2d day, 45 gr. KI given . . . .	15	
	3d day, 45 gr. KI given . . . .	14	
	Three days later . . . . .	15	
5	Control. Average for three days .	28	Saliva collected at 8.30 A. M., one and one-half hours after morning dose. Gradual increase in amount of KI in saliva as shown by starch test.
	1st day, 45 gr. KI given . . . .	53	
	2d day, 75 gr. KI given . . . .	40	
	3d day, 105 gr. KI given . . . .	50	
	Four days with no Ki given . .	31	
6	Control. Average for three days .	65	Large amount of KI in dilute saliva.
	1st day, 45 gr. KI given . . . .	80	
	2d day, 75 gr. KI given . . . .	75	
	3d day, 105 gr. KI given . . . .	110	
	Four days with no KI . . . . .	68	

In those experiments where 1 c.c. and  $\frac{1}{3}$  c.c. was added the accelerating influence is but little less than the experiment in which  $\frac{1}{2}$  c.c. was used. The small amounts of potassium iodide, therefore, act more powerfully than large amounts.

In Table II it is seen that in all these experiments, with the exceptions of numbers three and four, there is an increase in the amylolytic power of the saliva. In the experiments numbers three and four a decrease in the amylolytic power is seen. However, in these experiments there was no potassium iodide in the diluted saliva. The cause of this is not clear.

It may be that the potassium iodide had a marked systemic effect on these individuals and consequent decrease of the glandular secretions in general. This might explain the small amount of saliva excreted by the salivary glands, and also explain the lessened amylolytic power of the saliva in these two experiments. The small amount of potassium iodide excreted by the salivary glands in these two experiments may be due to the non-absorption of the potassium iodide by the alimentary canal.

The cause of the increased action of the ptyalin in the presence of potassium iodide may be due to a catalytic action of the potassium iodide, especially as small amounts accelerate the action of ptyalin quite as much as large amounts. It may be due to an increase in the concentration of the saliva and therefore a relative increase in the quantity of ptyalin.

## THE BEHAVIOR OF MUSCLE AFTER COMPRESSION.

BY LAWRENCE J. HENDERSON, G. A. LELAND, JR., AND  
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THE recent investigation of Henderson and Brink<sup>1</sup> has shown that muscular tissue is even less compressible than pure water, the diminution of its volume under a pressure of 500 atmospheres being not quite 2 per cent. This fact suggests that even great pressures, when gradually applied to muscle, may be without important effect upon its organization and contractility; accordingly the experiments here reported have been carried out to determine the behavior of muscle after compression.

For the studies freshly excised gastrocnemius muscles of the frog were used, and their condition before and after compression was determined by recording their response to maximum break induction shocks. Before stimulation, in every case, the muscle was loaded with a constant weight of 10 gm.; and thus the records consist of the ordinary isotonic curves of contraction.

*Experiment I.* — The muscles of two frogs, *A* and *B*, were removed and their response to stimuli recorded. One muscle (I) from each frog was carefully preserved moistened with normal saline solution, the other muscles (II), suspended in normal saline solution, were placed in the compression apparatus<sup>2</sup> previously described.<sup>3</sup> Here they were rapidly compressed to 500 atmospheres; at this pressure the system was maintained for one minute, whereupon the pressure was rapidly reduced. Next all four muscles were again stimulated and their responses recorded. Needless to say the response of the uncompressed muscles remained essentially unchanged. On the

<sup>1</sup> HENDERSON and BRINK: This journal, 1908, xxi, p. 248.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> For the use of the apparatus we are indebted to the kindness of Professor T. W. Richards and the Carnegie Institution of Washington.

other hand the compressed muscles were found to have been seriously damaged during compression. Muscle II of frog *A* showed no response to stimulus at any time after compression. Muscle II of frog *B* responded to stimulus, but only after a longer latent period than before compression, and with a weaker contraction, as is indicated by the following measurements taken from the records.

FROG B, MUSCLE II.

	Number of record.	Latent period. Seconds.	Height of curve. mm.
Before	{ 9	0.009	26
compression	{ 10	0.011	27
After	{ 11	0.014	2.0
compression	{ 12 <sup>4</sup>	0.015	1.2

*Experiment II.* — From a third frog, *C*, the muscles, I and II, were removed and their contractions recorded as before. They were then slowly compressed, as nearly as possible at the rate of 50 atmospheres per minute, and so far as possible without sudden increase of pressure, to 500 atmospheres, then immediately decompressed in the same way and at the same rate. Records from these muscles were taken immediately after compression, and again a half-hour later. The results are as follows:

FROG C.

	Muscle.	Number of record.	Latent period. Seconds.	Height of curve. mm.
Before	{ I	15	0.009	26
compression	{ II	16	0.008	25
Immediately	{ I	18	0.008	22
after	{ I	17	0.008	23
compression	{ II	23	0.017	8
	{ II	19	0.012	13
Thirty	{ I	21	0.007	23
minutes	{ II	20	0.013	8
later	{ II	22	0.015	10

Records 15 and 21 of the above table are here reproduced (Fig. 1). These records indicate, somewhat unexpectedly, that two like

<sup>4</sup> Record 12 was taken at a considerable time after record 11, and indicates no tendency to recover from injury.

muscles from the two legs of a frog may be very differently affected by precisely the same compression, when enclosed together in one vessel. Gradual compression to 500 atmospheres followed

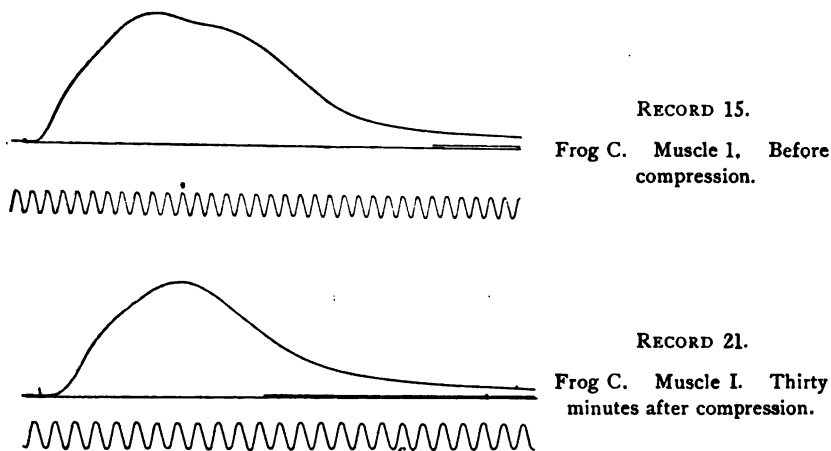


FIGURE 1. — One half the original size.

by decompression has weakened the contractions and lengthened the latent period of muscle II; the effect is, however, not so marked as in Experiment I.\* On the other hand muscle I has survived compression without material change in latent period or strength of contraction.

#### CONCLUSIONS.

These experiments prove that a pressure of 500 atmospheres gradually applied to a muscle and gradually released may be without material effect upon the response of the muscle to maximum break induction shocks. Probably therefore a pressure of 500 atmospheres is not in itself harmful to muscle tissue.

Accordingly it is not improbable that the observed harmful effects of compression in other cases were due either to too rapid compression and decompression, or, what amounts to the same thing, to irregularity in compression or in decompression.

### STUDIES IN RESUSCITATION.—III. THE RESUSCITATION OF THE GLANDS AND MUSCLES AFTER TEMPORARY ANÆMIA.<sup>1</sup>

By F. H. PIKE, C. C. GUTHRIE, AND G. N. STEWART.

[From the Hull Physiological Laboratory of the University of Chicago.]

#### THE RESUSCITATION OF THE GLANDS.

WE have previously considered, somewhat briefly,<sup>2</sup> the resuscitation of the salivary and other glands after anæmia. To the facts already given we may add the following. The salivary secretion may be maintained for a considerable time (one hour or more) with a mixture of defibrinated blood and Locke's solution<sup>3</sup> (see protocol of experiment of February 24, 1905). Pilocarpine (0.1 per cent solution), applied to the submaxillary gland two hours and twenty-seven minutes after starting the heart (occlusion of six and one-fourth minutes, heart stopped for about ten minutes beginning nine minutes after release), produced good secretion of saliva. At this time the secretory effect of the chorda tympani had returned, as shown by the copious flow of saliva on stimulation. Pilocarpine injected intravenously caused some secretion of saliva when the head end of the animal was perfused with a mixture of defibrinated blood and Locke's solution (experiment of March 1, 1905).

In one experiment (February 28, 1905) the effect of the chorda tympani upon the submaxillary gland disappeared when fresh defibrinated blood was circulated through the head of a dog, forty-three minutes after beginning the artificial circulation.

<sup>1</sup> The second of these studies on the "Reflex excitability of the brain and spinal cord" appeared in this journal, 1908, xxi, p. 359.

<sup>2</sup> STEWART ET AL.: *Journal of experimental medicine*, 1906, viii, p. 312.

<sup>3</sup> The protocols referred to in this paper are given in the "*Journal of experimental medicine*," 1908, x, p. 371.

Good secretion of saliva was observed three hours and forty minutes after release from a partial occlusion of fifty-one minutes. At this time the reflex excitability of the lower part of the spinal cord was high, and scratching movements of the hind legs were almost constant, but there were apparently no reflexes obtainable from the cervical cord. There was no response to striking the forelimbs, and no respiratory movements had occurred.

The secretion of tears often begins before the eye reflexes have been established. For example, twenty minutes after starting the heart by direct massage following a stoppage of fifteen to twenty minutes, tears were being secreted. At this time the pupils were beginning to contract, but respiration had not returned. Fifty-two minutes after starting the heart there was marked lachrymal secretion. The light and corneal reflexes and swallowing movements were back at this time. Thirty-six minutes after release, following an occlusion of twenty minutes in another experiment, touching the cornea caused the eye to water, but there were no movements of the pupil. The crossed reflexes of the forelimbs had also returned at this time. Two hours and forty-eight minutes after release, following an occlusion of forty-five minutes, the eyes were moist; three and one-half hours after release, the secretion of tears was active. Respiration never returned. In another experiment (May 22, 1905), after an occlusion of sixty minutes, the secretion of tears began three hours and forty minutes from the time of release. Bulbar respiration had not returned, and neither the corneal nor the light reflexes ever returned. Five hours after release both eyes were still moist. The rectal temperature at this time was  $28.7^{\circ}$  C. Eight hours after release the eyes were dry, but the corneal tension was good. The temperature, taken thirty minutes later, was  $32^{\circ}$  C.

In one experiment both eyes showed the presence of tears one hour and thirty-nine minutes after release from a partial occlusion of fifty-one minutes. The corneal tension at this time was good. Earlier in the resuscitation period there was evidence of some interference with the left cervical sympathetic, as the left pupil was much smaller than the right, and not larger than normal. Either the paralysis of the left sympathetic, possibly from injury, was not complete, or lachrymal secretion can be caused in some other way than through the cervical sympathetic.

Artificial circulation has not been an unqualified success in main-



taining the activity of the lachrymal apparatus. In one experiment (February 24, 1905) the spontaneous secretion of tears was lacking in the left eye, but present in the right. At this time, fifteen and one-half minutes after release, following an occlusion of four and three-fourths minutes, the corneal reflex was present in the right eye but not in the left. Later in the experiment, although there was still no spontaneous secretion of tears in the left eye, stimulation of the left vago-sympathetic nerve caused a copious secretion of tears,—the usual effect in the cat's eye. This effect afterward disappeared under the influence of the artificial circulating medium employed (eight parts 0.9 per cent sodium chloride plus one part defibrinated blood).

In an experiment (April 14, 1905) in which the head end of a pup was kept alive for a time by an artificial circulation of defibrinated blood, stimulation of the left vago-sympathetic trunk caused a good secretion of tears one hour after the beginning of the artificial circulation. Forty minutes later, and two minutes after stopping the artificial circulation, stimulation of the chorda tympani failed to cause any secretion from the submaxillary gland. Three minutes later, stimulation of the vago-sympathetic still caused dilation of the pupil and retraction of the nictitating membrane, but no lachrymal secretion.

In another experiment (March 1, 1905) in which a mixture of defibrinated blood and Locke's solution was circulated through the head end of a pup, pilocarpine caused good lachrymal secretion apparently only in the eye whose sympathetic nerve was intact. It is possible that here the secretory endings of the sympathetic had deteriorated under the influence of the circulating fluid.

In one experiment (March 4, 1905), in which an occlusion of ten minutes was combined with artificial circulation of defibrinated blood and Locke's solution through the head end of a cat, ammonia, applied to the nostrils one hour and twenty-three minutes after the release of the head arteries, or twenty-one minutes after the beginning of the injection of the artificial fluid, caused an increase in secretion from the nostrils. Respiration had begun two minutes before, and was now going on at the rate of nine in twelve and one-half seconds. Ammonia caused an increase in nasal secretion and in the movements of the face and whiskers in another similar combined experiment forty-six minutes after a second occlusion of six minutes at a time when no corneal reflex was present. These

facts indicate the resuscitation of the afferent fibres of the nasal branch of the fifth nerve.

In animals which recovered permanently after cerebral anæmia we must assume that the thyroid gland, which lies within the anæmic region during the occlusion of the cerebral arteries, has completely recovered its function, since such animals show no symptoms of athyroidea.

The fact that the lymph flow may persist for one to several hours during cerebral anæmia and in the period of resuscitation before any of the bulbar functions have returned, is evidence that no secretory or other nerves of bulbar or cerebral origin are necessary for the continued flow of the lymph. During such an experiment the blood pressure sinks to one third of its usual height, or even lower.

In the experiment of May 16, 1905, the head arteries were occluded for twenty-one minutes. Shortly after release the heart stopped, and was started by massage and clamping the aorta about ten minutes later. At autopsy five hours later, the urine was taken from the bladder and put in the ice chest over night. In the morning there was a copious, flocculent deposit, consisting largely of fat drops of various sizes, staining with osmic acid. The osmic acid caused them to lose their round outline and to become in many cases angular. The globules were soluble in ether. There were also numerous spermatozoa and many bladder epithelial cells, and groups of cells, including the tailed and faceted cells. The reaction of the urine was acid. The bladder epithelium might have been detached during the prolonged anæmia following clamping of the descending aorta, possibly from maceration of the dead bladder with the urine. If the fat globules came through the kidneys, they must have done so before the thoracic aorta was clamped, as it is not to be supposed that the kidneys would long continue to secrete in the absence of oxygen and with the blood pressure at zero. The vesiculæ seminales had evidently become lax during the long period of anæmia and allowed their contents to escape.

The urine which was taken from the bladder of a cat ten hours after its death, with the body at a fairly low temperature all of the time, and on ice during the last two and one-half hours, showed a decided amphoteric reaction. It gave a strong Trommer's test and the polarimeter showed a rotation to the right corresponding to exactly 3 per cent of glucose. The occlusion in this experiment

was thirty minutes, with death eight hours after release. Some ether had been given to control the spasms.

Exactly the same percentage of glucose had been found in another experiment a few days previous, in which the occlusion period was two and one-half hours but the anæmia only partial. The urine was passed during a severe spasm two and one-half hours after the ligatures about the arteries were first tightened. The fermentation test for glucose was positive.

#### THE RESUSCITATION OF THE MUSCLES.

The general question of the resuscitation of the muscles has previously been attacked from the point of view of the removability of rigor. Brown-Séquard,<sup>4</sup> after interruption of the circulation in the hind legs of a rabbit, found that the rigor induced by the anæmia disappeared when the circulation was re-established. Other authors have denied the existence of true rigor mortis in Brown-Séquard experiments.<sup>5</sup> In our own experiments we have found that the muscles of the head end have recovered their irritability after long periods of anæmia, but we have no decisive data on the removability of rigor, nor have we any direct experiments on the time which may elapse after apparent death before the muscle irrevocably loses its power to contract. In some of our experiments in which the aorta was clamped and the heart started by massage, the clamps were not removed until the autopsy. Five hours after clamping the aorta, the hind parts of the body were quite rigid, and had been that way for some time, although the heart was beating well up to that time. No attempt was made to remove the rigor by re-establishing the circulation through the posterior part of the body. The muscles have, in all experiments in which they have been subjected to the same conditions, been more easy to resuscitate than the nervous tissues, and we have seen no case of permanent paralysis following anæmia which could be shown to be due to muscular and not to nervous causes. For all practical purposes of resuscitation we may safely assume that the

<sup>4</sup> BROWN-SÉQUARD: Archives de physiologie normale et pathologique, 1889, pp. 675, 726; 1890, p. 628; 1894, p. 188.

<sup>5</sup> The literature of the *post mortem* duration of the irritability of the heart and muscles is given by ROTHBERGER (Archiv für die gesammte Physiologie, 1903, xcix, pp. 385-457).

muscles will withstand injurious influences in general much longer than the nervous system.

The muscles lose their excitability to direct as well as to indirect stimulation during anæmia, and recover this excitability after the return of the blood. The excitability to direct stimulation persists longer during anæmia, and returns earlier in resuscitation than that to indirect stimulation. The motor endings succumb more quickly than the muscles themselves.

In one experiment (May 16, 1905) the heart could not be felt twenty-one minutes after release from an occlusion of twenty-two minutes. The ventricles fibrillated for some time after clamping the aorta and starting the heart by massage, but suddenly started seventeen minutes after stoppage of the heart was first noticed. The total period of anæmia, either complete or partial, in this experiment must have been, therefore, nearly forty minutes. Five hours after starting the heart, stimulation of the peripheral end of the left vagus nerve nearly, but not quite, stopped the heart. No effect followed stimulation of the central end. The act of division of the right brachial plexus caused strong contraction of the muscles of the right forelimb. Stimulation of the central end of the brachial caused no effect. Stimulation of the phrenic nerve or of the diaphragm directly caused strong contraction of the diaphragm. These are instances of the greater resistance which, as Scheven<sup>6</sup> points out, the peripheral nerves and white substance, as compared with the gray matter of the central system, manifest toward anæmia. Respiration and the eye reflexes never returned. Stopping artificial respiration fourteen minutes later caused cessation of the efficient heart beats in less than one minute, although feeble beats persisted some two minutes longer. There were no spasms of any kind during the asphyxia.

Peculiar fibrillary contractions of the tongue are observed constantly at a certain stage in resuscitation. They occur too early to be due to stimulation of a motor nerve, and it was shown that excitation of the hypoglossal nerve was ineffective at a time when these fibrillations were very prominent. They are perhaps connected with a hyperexcitable condition of the muscle fibres, the complex arrangement of which in the tongue is doubtless responsible for the similarity of the movements to fibrillation of the heart muscle. The resemblance between these "boiling" movements of

<sup>6</sup> SCHEVEN: *Archiv für Psychiatrie*, 1904, xxxix, p. 169.

the fibrillating tongue and of the fibrillating heart is very striking. The twitching of the skin muscles of the shoulder and neck, which, as a rule, earlier than any other sign, heralds the return of function in the previously anæmic region, appears to be the form which this fibrillation takes in a less complex arrangement. These fibrillations come early after the return of the blood before the respiratory movements return. They cease later, and the tongue again moves in the normal manner.

An occlusion of six and one-fourth minutes was followed, about ten minutes after release, by a ten-minute stoppage of the heart. The heart was started by massage, but, owing to an accidental breaking down of the artificial respiration apparatus, the heart stopped again for about eight minutes. Twenty-six minutes after starting the heart a second time, the tongue began fibrillating slightly on the under side near the tip. The fibrillation was rapid and continuous, and was the first movement observed. Five and one-half minutes later (thirty-one and one-half minutes after starting the heart) the whiskers began twitching, the twitching increasing in three minutes. Thirty-six minutes after starting the heart the skin under the jaw began twitching. The movements spread to the skin of both cheeks and to the chin and corners of the mouth in three minutes more. The first respiratory gasps occurred forty-one minutes after release. At this time the fibrillary movements of the tongue were more vigorous than before, and involved the whole under side of the tongue as far back as could be seen conveniently, but were not seen on the dorsum of the tongue. The hypoglossal nerve was cut two hours and twenty-seven minutes after starting the heart, but the fibrillary movements of the tongue continued with undiminished intensity. Stimulation of the peripheral and of the hypoglossal caused strong contractions of the tongue. Artificial respiration was stopped two hours and forty-seven minutes after starting the heart. The respiratory gasps had not become frequent enough to maintain the oxygenation of the blood, and soon stopped. The fibrillary movements of the tongue stopped within a minute, although the tongue itself was still irritable to direct stimulation eighteen minutes after stopping the artificial respiration.

Occasionally the superficial muscles of the shoulder region begin twitching before the fibrillary movements of the tongue are seen. After a heart stoppage of about twenty-two minutes the circulation

was re-established by direct massage of the heart. Fifty minutes later the skin over the right shoulder began twitching. That the twitching was superficial (from the platysma myoides) was shown by cutting down and exposing the deeper muscles, which were quiescent. The tongue began twitching seven minutes, and the whiskers ten minutes, later. The muscles in the front of the neck have also been observed to twitch before the movements of the tongue began.

Twitching of the skin over the right hip, a region which had not been anæmic, was observed in one case thirty-six minutes after release from an imperfect occlusion of thirty minutes.

The twitching movements began as usual after release from an occlusion of forty-five minutes, but ceased, with the exception of a small area on the throat, eighty-five minutes after release. The throat movements ceased shortly afterward.

Occasionally only certain hairs of the mustache twitch. This was seen in one experiment, ten minutes after release from an occlusion of fifty minutes. The twitching then ceased for a time. The first gasp occurred sixty-seven and one-half minutes after release. Forty minutes later the mustache hairs were again twitching strongly.

#### THE SPASMS OF THE SKELETAL MUSCLES.

Little need be said in a general way of these here except that they originate in the central nervous system, that they do not appear until the brain and spinal cord have recovered, in some degree, their power of functioning, and either subside when the central nervous system has completely recovered, or terminate in the death of the animal. They cease in the parts below the plane of section when the spinal cord is transected. They differ in character from the fibrillations of the tongue, presenting, so far as we have observed, none of that boiling movement seen in the tongue.

Green<sup>7</sup> has suggested that these spasms of the skeletal muscles are analogous to the fibrillations of the heart. This is erroneous. A fibrillation of the striated muscle does occur, as has been said, but at a much earlier stage in the resuscitation than the spasms. The latter are undoubtedly of central origin, and may occur, as we have previously mentioned,<sup>8</sup> when the brain stem is divided at the upper border of the pons.

<sup>7</sup> GREEN: *Lancet*, 1906, ii, p. 1708.

<sup>8</sup> STEWART and PIKE: *This journal*, 1907, xx, p. 72.

The spasms have a fairly definite form, as a rule. Two hours after release from an occlusion of fifty minutes there was strong tonic spasm of the forelimbs, the jaws being flexed and the claws protruded, while the rest of the limb was rigidly extended. Clonic trembling movements were superposed on the tonic spasms, which lasted minutes at a time. Forty-three minutes after release from an occlusion of forty-five minutes each forelimb moved on striking it, but there was no crossing of reflexes. Clonic movements of the right forelimb, continuing almost without intermission, were going on at this time, at the rate of twenty-four in seven seconds, and (another observation) thirty in eight and four-tenths seconds. These clonic movements were immediately stopped by grasping the foot, or even by supporting it in the hand. Evidently a second stimulus inhibited the impulses giving rise to the clonic movements.

In another experiment there was a partial occlusion of twenty minutes, after which the ligatures were tied permanently and the wound sewed up. One hour later, there were strong tonic extensor spasms of the fore and hind limbs, and especially of the forelimbs. The hind limbs were strongly extended and the toes widely spread. The forelimbs executed clawing movements, as if pawing the air, and the right forelimb often executed circular sweeping or stirring movements. In the intervals between the spasms the toes of the hind feet were not spread. In another hour there was a spasmodic attack, which lasted more than two minutes. During the whole time the right forelimb executed scratching movements at the rate of sixty-one in thirty seconds, the claws being protruded. Just before the spasm ceased the claws must have been withdrawn, as the scratching sound of the foot on the table ceased several seconds sooner than the movements. The left forelimb did not participate in these movements, but was strongly extended throughout the attack. The hind limbs also were strongly extended, with the toes spread and the claws protruding. The animal passed urine, during the attack, in a strong stream. The attack passed off suddenly, and the limbs relaxed moderately. The breathing was very rapid after the spasm was over (28 in thirteen seconds). Eight minutes later another seizure of twenty seconds' duration occurred, during which the scratching movements had a peculiar double rhythm, two coming in quick succession, then a pause, and then two more.

The spasms, both tonic and clonic, are subdued by ether in moderate amounts. Severe spasms came on after release from an occlusion of thirty minutes. Seven hours after release the animal had been receiving ether for some minutes, and seemed fairly well under its influence. Both fore and hind limbs were, however, extended pretty stiffly except in the distal joints in each foot, which were slacker though not quite free from extensor spasms. Handling the hind limbs caused some contraction of them and quickening of respiration. Ether was given in small quantities for thirty-six minutes more. At the end of this time the hind limbs were relaxed in all joints, and free from all extensor spasm. The forelimbs were relaxed in the distal joints, but there was still extensor spasm in the proximal joint (upper arm). The middle joint (fore-arm) was more relaxed than the proximal joint. No movements were elicited on striking the limbs.

#### THE EFFECT OF ANÆMIA ON FŒTUSES *in utero*.

In a pregnant cat the uterus was opened about four hours after clamping the thoracic aorta. There were five embryos about one fourth grown. One of them was removed and the thorax opened. The heart was not beating. The auricles were filled with black blood, and the ventricles were empty. The heart and the skeletal muscles were totally inexcitable to electrical stimulation.

In another experiment, the protocol of which was given in our first paper,<sup>9</sup> the animal was allowed to live. Severe spasms occurred, lasting three and one-half days. On the twelfth day after the operation four kittens were born. One was dead when first seen, but the others were apparently normal in every respect. If any toxic substance had been formed in the maternal tissues during the convulsions, or by the degenerative processes occurring later in the central nervous system, the effect upon the kittens was not apparent. It is not probable that any severe spasms could have occurred *in utero* without more or less serious laceration of the foetal membranes, and it is equally improbable that any toxin in the maternal blood capable of producing convulsions of such extreme severity would be entirely without effect upon the foetuses *in utero*.

<sup>9</sup> Journal of experimental medicine, 1906, viii, p. 313.



# A QUANTITATIVE STUDY OF FARADIC STIMULATION.—I. THE VARIABLE FACTORS INVOLVED.

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## INTRODUCTION.

INDUCTION shocks have long been recognized by physiologists as constituting in many respects the most satisfactory agents for artificial stimulation of living tissues. Their high efficiency, ease of application and control, and absence of deleterious effect upon the tissues employed commend them for this purpose above other sorts of stimuli. There is, however, a serious defect in their use, arising from the lack of a satisfactory means of measuring their intensities. This lack will be felt more and more as physiological research tends to greater exactness. At present investigators are constantly employing stimuli whose absolute values are completely unknown and of whose relative strengths the most that can be said is that one is stronger or weaker than another. Even these gross differences cannot be stated with certainty when different induction mechanisms are in question. As a result it is difficult to compare the work of one investigator with that of another, or to reproduce exactly with different apparatus any experiment which involves the use of faradic stimuli. Experimenters are likewise handicapped in the course of single investigations by their inability to compare accurately the stimuli used under different conditions, or to set their apparatus beforehand so that it will give stimuli whose values will bear a certain ratio to those used at other times. These drawbacks to the highest usefulness of faradic stimuli would disappear if there were available a method for stating strengths of stimuli directly in units; these units to take into account all the variable factors which enter into the production of induction shocks and to express their resultant. Such a system would sim-

plify the description of experiments, increase their value by making them exactly comparable, and also open a field for further investigation of the quantitative relationships existing between stimuli and responses under various conditions.

The purpose of this research was to study the different factors involved in the production and use of faradic stimuli in order to see whether they can be so controlled as to permit the development of a system of units by which to express the values of their resultants, and to learn whether such a system can be made simple enough to be generally applicable. In this and succeeding papers the results of the study of these problems will be presented. For much valuable aid and many helpful suggestions in connection with the physical part of the work, I wish to acknowledge my indebtedness to Professors George W. Pierce, Wallace Sabine, and B. O. Peirce of the Jefferson Physical Laboratory of this University, and also to Professor C. M. Smith of the Department of Physics of Purdue University.

#### THE VARIABLE FACTORS INVOLVED.

It is obvious that the intensity of any induction shock must be dependent upon the construction of the inductorium by which it is produced, this construction involving the number of windings in primary and secondary coils and the dimensions of each, as well as the form and magnetic nature of the iron core; also upon the intensity of the current which is made or broken through the primary coil, and upon the position of the secondary with respect to the primary. It is also well known that make shocks differ in intensity from break shocks. Most observers have noted in their own experience what Helmholtz<sup>1</sup> pointed out, that the sort of key which is used in opening and closing the primary circuit, and the way in which it is handled, have much influence upon the intensity of the secondary shock. Finally the tissues which are subject to stimulation present wide differences in electrical resistance, and since they form part of the secondary circuit, must affect the currents generated therein. There are, then, the following five subjects to be considered in developing a scheme for measuring faradic stimuli: the inductorium; the relation between make and break

<sup>1</sup> HELMHOLTZ: POGGENDORF's *Annalen der Physik und Chemie*, 1851, lxxxiii, p. 505.

shocks; the contact key; the primary current; the secondary resistance. In this paper the problems will be stated and some introductory comments made. The chief observations will be presented in succeeding papers.

#### THE INDUCTORIUM.

Faradic stimuli, other conditions remaining constant, are functions of the construction of the induction coil and the position of the secondary relative to the primary. Since the former is a constant factor, its value can be determined once for all. Inasmuch as it is the usual practice of physiologists to vary the strength of stimulus by varying the position of the secondary coil relative to the primary, a scheme for measuring stimuli must include a scale in which the influence of this factor has been worked out. Such scales have been prepared by Fick<sup>2</sup> and Kronecker,<sup>2</sup> those of the latter being in common use at the present time, and representing the furthest point of present attainment in the direction of measuring induction shocks. It is in order, then, to examine the Kronecker graduation with a view to determining its availability for the purposes of this study.

The Kronecker system of units, as is well known, is in principle nothing more nor less than an empirical expression of the relative deflections of a galvanometer needle caused by the current induced in the secondary coil at the different points on the scale, when a constant current is made or broken through the primary. In preparing this scale, moreover, Professor Kronecker<sup>3</sup> considered it advisable to remove the iron core from the primary coil. There are two questions which arise in connection with an examination of the Kronecker scale: 1. Do the physiological effects of induced currents at different positions of the secondary coil bear to one another precisely the same ratio as the galvanometer deflections for those positions? 2. What effect does the presence of the iron core have upon these ratios?

With regard to the first question Helmholtz<sup>4</sup> showed that within certain limits the physiological effects of *break* induction shocks

<sup>2</sup> The method of preparing these scales is given by CYON: *Methodik der physiologischen Experimente*, Giessen, 1876, pp. 379 *et seq.*

<sup>3</sup> CYON: *Loc. cit.*, p. 381.

<sup>4</sup> HELMHOLTZ: *Loc. cit.*

do vary as the galvanometer deflections caused by the same induced currents. To this extent, therefore, the Kronecker scale might avail for the present purpose, provided the induction apparatus be used without the iron core in place. Since, however, the common practice is to use the inductorium with the iron core present, the second question becomes important. In order to answer it satisfactorily the writer had recourse to an experiment

TABLE I.  
EXPERIMENT OF APRIL 6, 1907. BREAK SHOCKS.

Position of secondary coil. Kronecker scale.	Primary current amperes.	Product of current times scale reading.	Author's constant.
12000	0.0021	25.2	8.02
9000	0.0025	22.5	8.24
6000	0.0032	19.2	8.06
3000	0.0059	17.7	8.20
2000	0.0088	17.6	8.54
1000	0.0158	15.8	8.37
400	0.0355	14.2	8.44
200	0.065	13.0	8.58
100	0.115	11.5	8.62
50	0.215	10.8	8.78

the result of which is given in Table I. The experiment was based upon a fact demonstrated by Helmholtz,<sup>5</sup> that within certain limits the stimulating effects of break-induction shocks vary directly as the intensities of the primary current. The method of work was as follows: The intensity of primary current necessary to produce a break shock of a certain strength<sup>6</sup> was determined for each position of the secondary on the Kronecker scale. These values are given in column two of the table. Since they represent the same stimulus throughout, evidently the efficiency of the secondary as it is pushed away from the primary must fall off at the

<sup>5</sup> HELMHOLTZ: *Loc. cit.*

<sup>6</sup> The method of measuring the strength of stimulus is given in the second paper of this series (p. 117 this number).

same rate as the current through the primary increases. If the Kronecker graduations represent truly these relative efficiencies, the products of the several scale divisions by their corresponding primary currents should give a constant. In column three these products are set down, and, as will be seen, they show no tendency to be constant. In the fourth column of the table are given the constants obtained for this same experiment by a method to be described in an accompanying paper (p. 131). These are introduced here to show that there was no considerable error in determining the primary currents, such as would account for the failure of the Kronecker graduations to give constants. It is clear, then, that the Kronecker scale does not give a true picture of the influence of the position of the secondary upon the intensity of stimulus in an inductorium whose iron core is in place.

While there is abundant theoretical justification for the omission of the iron core, especially where quantitative estimations are sought, for the practical purposes of the physiologist the inductorium as commonly used, with the iron core present, is to be preferred. The intensity of stimulus for any given primary current is at least five times greater with the iron core than without it in inductoria of the usual Kronecker type. This increased efficiency makes it possible to obtain with primary currents of moderate intensity as strong stimuli as the physiologist ordinarily requires. The use of moderate primary currents is of great importance in quantitative estimations of induction shocks, since thereby is avoided that heavy sparking at the contacts which always accompanies the break of a current of high intensity, and whose effect upon the intensity of the stimulus, while very marked, cannot be foretold.

When the secondary coil of an inductorium is moved along from the zero position until it is nearly clear of the primary coil, it enters a "critical region" where small changes in position are accompanied by great changes in the intensity of the stimuli given by the instrument. The impression seems to prevail among physiologists that inductoria having iron cores show so much greater variations of intensity in this "critical region" than do those without iron cores as to make the omission of the iron core a distinct advantage in many experiments. As a matter of fact, however, the Kronecker inductoria used in the present research show for given changes in secondary position in the "critical region" greater

variations in stimulation intensity with cores removed than with cores present. This became first apparent when the Kronecker graduations of these coils were compared with the calibrations made for them in connection with the present work (see p. 130). In the preparation of the Kronecker graduations the iron cores were

TABLE II.  
EFFECT OF THE IRON CORE ON THE RATE OF CHANGE OF STIMULATION INTENSITY  
IN THE "CRITICAL REGION" OF THE INDUCTORIUM.

Position of secondary in centimetres.	Iron core absent.		Iron core present.	
	Kronecker graduation.	Percentage decrease per centimetre.	Author's calibration.	Percentage decrease per centimetre.
8	6190	....	2600	....
9	5150	17.0	2240	13.8
10	4150	19.4	1880	16.1
11	3250	21.7	1500	20.2
12	2375	27.0	1100	26.7
13	1570	33.9	800	27.2
14	1000	36.3	530	33.8
15	625	37.5	360	32.1
16	435	30.4	250	30.6
17	310	28.7	190	24.0
18	230	25.7	145	23.7
19	178	22.7	116	20.0

withdrawn from the instruments. For the calibrations made in connection with this work the iron cores were in place.

Table II gives a comparison of the calibrations in the "critical region" of one inductorium made without and with the iron core. The primary coil of this instrument was 14 cm. long. The table shows clearly that the rate of decrease of stimulation intensity from point to point is greater when the iron core is absent than when it is present. Table III is the record of experimental verification of the same fact. Stimulation intensities were compared

in this experiment in the same way as in the experiments cited in Table I (p. 64), namely, by comparing the primary currents required to produce stimuli of equal value with the secondary coil at different positions. According to this method increases in the primary current represent corresponding decreases in the stimulating efficiency of the inductorium.

TABLE III.

EXPERIMENTAL PROOF THAT STIMULATION INTENSITY SHOWS GREATER VARIATION IN THE "CRITICAL REGION" WHEN THE IRON CORE IS ABSENT THAN WHEN IT IS PRESENT. BREAK SHOCKS.

Date of experiment.	Position of secondary in centimetres	Iron core absent.		Iron core present.	
		Primary current amperes.	Percentage increase in current.	Primary current amperes.	Percentage increase in current.
Dec. 21, 1906	8.0	0.0195		0.00187	
	12.0	0.0505	159	0.00463	148
	16.0	0.260	415	0.022	375
Dec. 24, 1906	8.0	0.00576		0.0008	
	12.0	0.01523	164	0.00197	146
	16.0	0.091	432	0.00934	374
April 15, 1907	8.2	0.017		0.0036	
	11.28	0.035	107	0.0063	75
	12.45	0.0535	50	0.0092	46
	14.0	0.107	100	0.016	74
	16.2	0.2485	132	0.034	112

The chief practical objection to the use of the iron core in quantitative work is that changes of temperature affect the magnetic nature of the iron, and hence the influence of the core upon its surrounding coils. As a matter of fact, however, the variations of magnetic property for the range of temperature found in physiological laboratories are so slight as to be negligible. Inasmuch as the use of the inductorium without the iron core is in practice more objectionable than its use with one, the inductorium with the core is to be preferred if a suitable scale corresponding to the

Kronecker graduation can be prepared for it. An accompanying paper (p. 116) shows that the labor of preparing such a scale is no greater than is required for the preparation of the Kronecker scale. The Kronecker units, moreover, leave something to be desired in that they are wholly empirical, giving relative values under the limitations stated above, but no suggestion at all as to the actual physical values of the stimuli obtained. The purpose of this and following papers is to develop a scheme for determining relative values, and also, if possible, to make the units by which these values are expressed such as to give definite information of the physical values of the stimuli used.

#### THE RELATION BETWEEN MAKE AND BREAK SHOCKS.

The marked difference in intensity usually existing between make and break shocks produced under uniform conditions is commonly accounted for by the statement that the extra current through the primary due to its self-inductance is more effective in diminishing the inductance in the secondary on the make than on the break.<sup>7</sup> According to the usual explanation,<sup>8</sup> the extra current at the break is less effective than at the make because the interruption of the primary circuit prevents the passage of the extra current, save for that part of it which appears as the spark. This explanation is in accord with the statement of Helmholtz,<sup>9</sup> that anything which increases the sparking at the contacts diminishes correspondingly the intensity of break stimuli. The early users of induction shocks were particularly impressed by the influence of this factor and laid great emphasis upon it, with the result that present-day physiologists have not had called to their attention the fact that the differences between make and break shocks really depend upon several factors, of which the extra current due to self-inductance in the primary, although important, is only one.

The first additional factor to be mentioned is the number of turns in the secondary coil. As Table IV shows, there is a varia-

<sup>7</sup> ROSENTHAL: *Allgemeine Physiologie der Muskeln und Nerven*, Leipzig, 1899, p. 299.

<sup>8</sup> STEWART: *University of Pennsylvania medical bulletin*, February, 1904, p. 5.

<sup>9</sup> HELMHOLTZ: *Loc. cit.*; also *Philosophical transactions*, May, 1871, series iv, xlii, p. 232. See also FLEMING: *The alternate current transformer*, London, 1892, i, p. 189.



tion in the ratio of make intensity to break intensity when the number of turns in the secondary is changed, the direction of this variation being toward a relative increase in the break intensity as the number of turns increases. For the experiment cited in the table a secondary coil was constructed in three sections, each section containing one thousand turns of fine wire, the different sections being superposed. The sections were connected in series so that one, two, or all three together could be used. The comparison of make and break shocks was made by determining the primary currents necessary to give equal stimuli<sup>10</sup> in the different

TABLE IV.  
EXPERIMENT OF SEPTEMBER 30, 1907. SECONDARY AT ZERO.

No. turns in secondary.	Primary current. Makes.	Primary current. Breaks.	Ratio break currents to make currents.
1000	0.0031 ampere	0.00375 ampere	1.21
2000	0.0020 "	0.00235 "	1.17
3000	0.00162 "	0.0016 "	0.99

cases. Since, as the table shows, the relative amount of current needed for a given make stimulus increases when the number of turns in the secondary is increased, it follows that the efficiency of make stimuli falls off relatively in the same proportion.

A second additional condition affecting the relative intensities of make and break shocks is the position of the secondary coil with respect to the primary. As the two are moved farther apart, the ratio of make shock to break shock diminishes. A demonstration of this fact is cited in Table V. The method of comparing make and break stimuli was the same as above.

Still another condition which affects the relation under consideration is the intensity of the primary current. Beyond a certain minimum and up to a certain maximum the ratio of make to break diminishes as the primary current increases. This effect is due to the magnetization of the iron core, and its further discussion will properly come in the section devoted to that phenomenon.

A review of the preceding paragraphs shows that the relation between make and break shocks depends upon four factors: the

<sup>10</sup> See footnote, p. 64.

extra current in the primary coil, the number of turns in the secondary coil, the position of the secondary relative to the primary, and the magnetization of the iron core. Of these factors, one, the number of turns in the secondary coil, is fixed for each instrument and requires only to have its influence determined once for all. The method of taking into account the influence of the magnetization of the iron core will be discussed in a lower paragraph. There remains to consider the influence of primary self-inductance and of the position of the secondary coil with respect to the primary. It is possible by the use of suitable apparatus to minimize the

TABLE V.  
EXPERIMENT OF APRIL 18, 1907.

Position of secondary in centimetres.	Current through primary. Makes.	Current through primary. Breaks.	Ratio break currents to make currents.
0	0.0018 ampere	0.00126 ampere	0.70
16	0.0297 "	0.0203 "	0.68
20	0.11 "	0.0573 "	0.52
24	0.335 "	0.113 "	0.34

sparkling at the contacts, thus rendering the influence of primary extra currents on break shocks practically negligible. Make shocks, on the other hand, are profoundly influenced by whatever conditions affect the self-inductance of the primary, and it is therefore necessary to take account of all such conditions in developing a method for estimating make stimuli.

Since there are conditions affecting one sort of stimuli and not the other, and also in view of the fact cited above that the ratio of make shocks to break shocks changes with variations in the position of the secondary relative to the primary, it is evident that no single calibration will suffice for both kinds of stimuli. Break shocks require a calibration in which the effects of the particular factors influencing their intensities are worked out; and make shocks, being influenced by different factors, require different calibration. When proper calibration tables have thus been prepared, it should, however, be possible to make them comparable by the

use of suitable reduction factors. The method adopted in the present work is along the line here suggested.

#### THE CONTACT KEY.

Enough has been stated in previous paragraphs to show that the influence of the contact key upon the strengths of stimuli is very great. Helmholtz<sup>11</sup> emphasized the wide variations in intensity which accompany the use of different keys or even the same key differently handled. Since these variations are subject to no law that can be worked out beforehand, it is obvious that the only course to pursue is to eliminate them so far as possible. This can be done to a considerable extent by the use of primary currents of only moderate intensity,<sup>12</sup> thereby avoiding excessive sparking, and by making the contacts through a key constructed especially with a view to uniformity of action. Since sparking cannot be entirely eliminated except by the introduction of condensers, which in turn would bring in a factor impossible of measurement, and since two keys giving exactly similar sparks would be scarcely ever found, it is necessary to calibrate whatever key is used in terms of some selected standard.

#### THE PRIMARY CURRENT.

The effectiveness of an induction shock is of course closely dependent upon the primary current whose make or break generates it. It is therefore essential that the primary currents be taken into account in quantitative estimations of the value of induction shocks. The inclusion of this factor is a very simple matter so far as break shocks are concerned, since, as Helmholtz<sup>13</sup> showed, the stimulating effects of break-induced currents vary directly with the intensity of the primary currents, and are independent of their E. M. D. P.

There are certain conditions, however, under which the law laid down by Helmholtz for break shocks does not hold exactly.

<sup>11</sup> HELMHOLTZ : POGGENDORF'S *Annalen*, *loc. cit.*

<sup>12</sup> What constitutes a primary current of moderate intensity depends upon conditions. With the apparatus used in this research 0.75 ampere was the strongest current employed.

<sup>13</sup> HELMHOLTZ : *Loc. cit.*

These conditions arise from the presence of the iron core in the primary coil; and to meet them, a simple correction method must be applied. When the primary current rises above a certain intensity, — in the inductoria used in this work 0.1 ampere, — it begins to produce an appreciable magnetization of the iron core. The effect of this is to increase the efficiency of the break shocks, making them stronger than primary currents of the intensity used would be expected to produce according to the statement of Helmholtz. The amount of magnetization in any given iron core for different primary currents can be readily determined experimentally, according to a method to be described in the succeeding paper, and a simple correction formula deduced.

Determination of the influence of the primary current upon the strengths of make stimuli is less simple than for break stimuli, since, as will be shown in a later paper, the strengths of make shocks depend on the voltage of the primary current as well as upon its intensity. It is necessary, therefore, not only that the voltage of the primary current be known when make shocks are to be measured, but also that the scheme of calibration for make shocks shall have taken into account the influence of the voltage.

#### THE SECONDARY RESISTANCE.

The secondary circuit of an apparatus for faradic stimulation includes, in addition to the secondary coil, the living tissue undergoing stimulation, — an element whose electrical resistance is high, usually mounting into thousands of ohms, and, from the nature of the case, subject to wide variations. The problem here is to learn just what is the relationship between the strength of stimulus and the resistance in the secondary circuit, provided such a relationship exists. It is well known that the momentary current which is induced in a secondary coil when a current is made or broken through its primary varies as a whole inversely as the secondary resistance. This is shown by the changes in deflection of a ballistic galvanometer when the secondary resistance is varied. Helmholtz<sup>14</sup> pointed out that the physiological effect of an induced current is apparently all exerted during the first stage of its production. He also showed that with break shocks this stage is practically independent of the resistance in the secondary circuit.

<sup>14</sup> HELMHOLTZ: *Loc. cit.*

Helmholtz' discussion of this point will be presented in detail in an accompanying paper (p. 118).

In a paper to be devoted to the measurement of make shocks it will be shown that there are both theoretical and experimental reasons for concluding that the resistance in the secondary circuit has a marked influence upon the strengths of these stimuli. It is therefore necessary for the estimation of make shocks that the resistance of the tissue in the secondary circuit be known.

#### SUMMARY.

1. The usefulness of faradic stimuli would be enhanced if there were available a method of determining their values simply and accurately, and expressing the same according to a system of units.

2. The preparation of such a system involves a consideration of all the variable factors which enter into the production of faradic stimuli. These are: the inductorium; the relation between make and break shocks; the contact key; the primary current; the secondary resistance.

3. Since induction coils vary in construction, each must be calibrated in terms of some standard, and a scale made showing the effect of varying the position of the secondary relative to the primary. The Kronecker graduation does not suffice for this in coils used with iron core in place.

4. To make all sorts of faradic stimuli available, the values of both make and break shocks must be determined. Each requires its own inductorium calibration, since the two do not vary together.

5. The intensities of faradic stimuli depend to a large extent upon the nature of the contact key used. In order to make them comparable, keys must be employed which give constant results and which have been calibrated in terms of some standard.

6. The strength of a break stimulus varies in general directly as the intensity of the primary current. When the latter is large enough, however, to produce an appreciable magnetization of the iron core, this relation does not hold strictly, and a correction must be applied.

7. The strength of a make stimulus varies with the intensity and also with the voltage of the primary current.

8. Although the secondary resistance is very variable because

of the wide variations in resistance which living tissues exhibit; it appears that it need not be taken into account in determining the strengths of break stimuli, because the value of that part of a break induced current which has a physiological effect seems to be independent of the secondary resistance.

9. In order to measure make shocks the resistance of the secondary circuit, which includes the tissue under stimulation, must be known.

#### CONCLUSION.

If the values of faradic stimuli are to be expressed by a system of units, each unit will have to comprise the following factors: (1) One which shall state the efficiency of the particular inductorium used in terms of a standard, and also the efficiency of the secondary in the position it occupies relative to the primary, this factor being different for make and break shocks; (2) One which shall give the intensity of the primary current, corrected for voltage when make shocks are used and, if necessary, for the magnetization of the iron core when break shocks are to be measured; (3) One which shall state the effect of the contact key used in terms of a standard; (4) A reducing factor for bringing make and break shocks to common terms, made necessary by the fact that they are measured by different calibrations.

## THE RELATION OF IONS TO CONTRACTILE PROCESSES.—III. THE GENERAL CONDITIONS OF FIBRILLAR CONTRACTILITY.

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IN the second paper of this series<sup>1</sup> an attempt was made to explain the influence of calcium salts in furthering mechanical inhibition in the Ctenophore swimming-plate. A general theory of fibrillar contractility, suggested in the main by the conditions observed in this structure, was then outlined, which in brief was as follows. Contraction is directly due to a coagulative change in the colloids composing the contractile fibrillæ. The determining condition of this coagulation is a sudden increase in the permeability of the limiting membrane of the contractile element; in consequence of this change a transfer—or possibly interchange—of ions takes place between the interior and the exterior of the element. The ionic content of the fibril being thus suddenly altered, an aggregation change follows; this last event, coagulative in nature, forms the direct condition of the contraction, and is reversed during the relaxation phase of the beat. The essential facts brought forward in support of this view were of three kinds: First, the visible connection between coagulative changes and contraction in the swimming-plate; this is a conspicuous feature of the abnormally accelerated movement seen in many salt solutions. Second, evidence that the required interchange or transfer of ions, implying increased permeability of the surface layers, is in fact a constant correlate of contraction, is seen in the electromotor change known as the action current, which we have every reason to believe is an inseparable accompaniment of all forms of stimulation and motor

<sup>1</sup> R. LILLIE: This journal, 1908, xxi, p. 200.

activity. The justification of so broad a statement will be admitted by all physiologists. Third, the aggregation state of colloids is known to vary in a definite manner with variations in the ionic content of the colloidal system. It may therefore be held that the fundamental assumptions of the above theory are fully in agreement with well-established facts.

Several questions were left unconsidered in the paper cited, especially those relating to the exact conditions of the coagulative change in the fibrils, — why, for instance, it should be coagulative rather than in the reverse direction. The chief further problems to be considered are: (1) To what degree does the contractile process in the swimming-plate correspond to that existing in other contractile tissues and particularly in muscle? (2) What is the precise mode of action of the various stimuli, — chemical, mechanical, electrical, and nervous, and in what respects are they identical in nature? (3) How is the chemical energy of the energy-yielding compounds in the tissue transformed into the mechanical energy of contraction? Various accessory questions naturally relate themselves to these. In the present paper I propose to give a fuller but still concise account of the above theory, with certain modifications; and without adducing many new facts to consider the phenomena of contraction in the light of our present more complete knowledge of the colloidal state, and particularly in the light also of the now widely advocated “membrane theory” of the origin of electromotive phenomena in organized tissues. This theory, originally suggested by Ostwald<sup>2</sup> and first definitely advocated by Bernstein,<sup>3</sup> has furnished by far the clearest and most consistent explanation which we possess of these fundamental and hitherto enigmatic phenomena. Since electromotive changes invariably accompany contractile activity and the two apparently diverse phenomena are obviously closely interdependent, it is clear that any theory of contractility must account for this constant connection. Such a theory must therefore start from the fundamental and fully established facts: (1) the existence of a “physiological polarization” at the surface of the resting contractile elements, the outer surface being positive relatively to the inner, and (2) the diminution or disappearance of this polarization (the “negative variation”

<sup>2</sup> WILHELM OSTWALD: *Zeitschrift für physikalische Chemie*, 1890, vi, p. 71. Cf. especially § 16, p. 80.

<sup>3</sup> BERNSTEIN: *Archiv für die gesammte Physiologie*, 1902, xcii, p. 521.



or action current) during activity, — whether spontaneous, as in the heart, or due to outside stimulus. A connection must be shown to exist between these changes and the coagulative change which, according to the above theory, occurs in the fibrillar colloids during contraction and forms the condition of the mechanical effect.

Let us consider, then, the case of a simple contractile element such as a muscle cell or a cilium. The protoplasm of such a structure shows a differentiation into a system of longitudinal parallel fibrils separated by an interstitial non-fibrillar "sarcoplasm." The entire structure is surrounded by a modified surface layer or plasma membrane, apparently a complex of lipoid and protein material, as indicated by definite peculiarities of permeability. This surface layer shows during life a characteristic electrical polarization, so that the cell is positive externally and negative internally, — a peculiarity which, according to the Ostwald-Bernstein theory, depends on a difference of permeability relatively to anions and cations. The membrane is permeable to certain of the intracellular cations — exactly which is at present not finally determined — and impermeable to anions; the cations therefore leave the cell until their osmotic pressure is balanced by the electrostatic stress thus arising. The condition is, in fact, closely comparable to that of a metallic plate of high solution tension immersed in a solvent. Thus a condition of equilibrium is reached, stationary so long as the cell is undisturbed (or unstimulated), with outer positive and inner negative surfaces. This condition is the *physiological polarization*, which there is every reason to believe is characteristic of most if not all living cells.<sup>4</sup> Since it is conditional on the characteristic differential permeability relatively to the two kinds of ions, it is abolished by any conditions which alter the plasma membrane so as to render the latter permeable to anions. The peculiarity is found only in *living* cells, and disappears or is greatly diminished with death; hence the long-recognized rule that dead or dying portions of a cell are negative relatively to those still living. The physiological polarization may also be abolished by local injury through mechanical or chemical means, or by burning the cell surface; hence such injured regions are negative relatively to uninjured.

A similar diminution or loss of the physiological polarization —

<sup>4</sup> Cf. BERNSTEIN: "Elektrische Eigenschaften der Zellen und ihre Bedeutung," *Naturwissenschaftliche Rundschau*, 1904, xix, p. 197; BRÜNNING: *Archiv für die gesamte Physiologie*, 1903, xcvi, p. 241, and c, 1903, p. 367.

*i. e.*, a *depolarization*, which may vary in degree — accompanies stimulation and is evidently an indispensable condition of this process. Stimulation, then, follows when the resting polarization is diminished or removed. Mechanical agencies, by stretching the plasma membrane, increase its permeability and allow anions to pass; so also in the case of other forms of stimulation that directly alter the surface layer of the cell — as chemical or thermal stimuli. With electrical stimuli no such direct alteration of the membrane appears to take place, — since prolonged repetition of such stimuli may have no injurious effect, — but at the moment of making and at that of breaking the current a temporary depolarization results, — in the former instance at the cathode, in the latter at the anode, — in other words, wherever the surface positivity is lowered by the change in the outside current. The characteristic law of polar stimulation thus becomes intelligible. During the passage of the current the normal physiological polarization evidently re-establishes itself, since the tissue remains unstimulated; the stimulating current thus does not act by the introduction of ions into the cell, but merely by affecting its surface polarization at the times of sudden changes in intensity. A corollary of this consideration is that a change in current intensity, in order to act as stimulus, must exceed in rapidity the rate at which the surface polarization is established; hence a change in current intensity must be rapid in order to stimulate. In the living organism depolarization is usually initiated in the nerve, whence it is transmitted to the muscle; the nerve impulse on this view is essentially a *wave of surface depolarization*<sup>5</sup> which traveling along the nerve passes over to the surface of the muscle at the motor end plate, where nerve and muscle become continuous. The problem of nerve conduction thus relates essentially to the conditions under which such a state of depolarization is transmitted along the surface of the conducting element, whether nerve or muscle. The conditions in the nerve, where the impulse has an unusually high propagation velocity, must naturally exhibit some special peculiarities. But the stimulating effect, with which alone we are at present concerned, depends simply on the transmission of a condition of depolarization to the muscle surface.

Increasing the permeability artificially, as by mechanical stimu-

<sup>5</sup> Cf. BRÜNINGS: Archiv für die gesammte Physiologie, 1903, c, p. 367; the relation of depolarization to stimulation is discussed very interestingly in this paper.

lation or local injury, removes the polarization, as already seen; and conversely, removing the polarization by electrical means increases the permeability, so that anions then pass and stimulation results. It is noteworthy that the depolarized condition cannot in the living cell be long maintained; the physiological polarization is automatically re-established at once. Hence depolarization at the cathode on making a current is only momentary, and the normal polarization is quickly regained and remains constant during the unaltered flow of the current; the condition of equilibrium is, however, different from that existing previously to the passage of the external current, and the external excess of cations is partly maintained at the anodal region by this current. When the latter is broken, a temporary depolarization occurs therefore in this region, — hence the anodal stimulation at the break. The following general conclusions follow directly from these considerations: (1) in the resting element the peculiar differential permeability is *dependent* on the surface polarization, since it disappears when this is diminished or removed; and (2) this condition tends automatically to be re-established whenever disturbed, — *i. e.*, it is the normal condition of equilibrium in the living and inactive cell. Hence a rapid succession of stimuli — *i. e.*, a rapid alternation of polarization and depolarization — is necessary in order to produce continued contraction in a muscle. The exact conditions that determine this remarkable peculiarity are at present imperfectly known. That the changes of permeability depend on alterations in the colloidal consistency of the plasma membrane seems highly probable, as indicated particularly by Hoeber's researches;<sup>6</sup> the permeability must thus change with changes in the ions in contact with the membrane; and we can only assume that the presence of an external layer of cations and an internal of anions is a necessary condition of the distinctive vital permeability of cells. Why this condition is so readily and rapidly re-established when disturbed remains to be determined. A high velocity of the cation relatively to that of the anion seems to be indicated: if the penetrating cation is the hydrogen ion, as suggested below, the rapid re-establishment of the polarized condition is less difficult to explain, since the advancing layer of the outwardly diffusing electrolyte would be then positively

<sup>6</sup> Cf. especially HOEBER: *Archiv für die gesammte Physiologie*, 1907, cxx, p. 492; also *ibid.*, cvi, 1905, p. 599; and *Physikalische Chemie der Zelle und der Gewebe*, 1906, 2te Aufl., pp. 272 *seq.*

charged because of the high migration velocity of this cation. Apart, however, from all attempts at explanation, the fact remains that the physiological polarization, with outer surface positive, tends to be regained very rapidly after disturbance, so that the effect of a single stimulus is only momentary.

The above considerations, while mainly corollaries of the membrane theory as developed or advocated by Bernstein, Brünings, and Hoeber, are necessary as a preliminary to the more special consideration of the nature of contraction. Assuming at the outset that contraction is due to a coagulative change in the fibrillæ — a thesis for which I have already given direct evidence in the case of the swimming-plate<sup>7</sup> — the question becomes: Why should surface depolarization lead to coagulative changes of this kind? As Brünings puts it, muscle cells are so constructed that depolarization of the plasma membrane leads to a definite form alteration.<sup>8</sup> The question is: Why does the coagulative change, the condition of the form alteration, result from such depolarization?

Two fundamental considerations must be borne in mind here. The first is that the cytoplasmic colloids, including those of the contractile fibrillæ, are undoubtedly negatively charged during life. This follows from the known chemical nature of the proteids of such cells, — myosin, cell globulins, etc., in which the acid characteristics are more pronounced than the basic; it is also shown by the affinity of the cytoplasmic colloids for the basic *intra vitam* dyes (methylene blue, neutral red, etc.) in which the chromophore group forms the cation. Now, it is sufficiently clear, from all of the recent work on the action of electrolytes on colloidal solutions, that anions and cations have opposite actions on such colloids, — anions tending to promote a fine state of subdivision, *i. e.*, have a liquefactive or dissolving influence, while cations are coagulative.<sup>9</sup> The effect of any electrolyte is due to the sum of these two opposed actions. The inference is that a coagulative change in the cell (assuming the change to be due to ions) signifies an ascendancy of the influence of cations. We must therefore ask, in the terms of our hypothesis: Through what means do the cations gain such a tem-

<sup>7</sup> R. LILLIE: This journal. 1906, xvi, p. 117.

<sup>8</sup> BRÜNINGS: *Loc. cit.*

<sup>9</sup> With regard to the opposite actions of anions and cations on protein solutions, *cf.* PAULI: Beiträge zur chemischen Physiologie und Pathologie, 1902, iii, p. 225; also MATHEWS: This journal, 1905, xiv, p. 203, especially pp. 217 *seq.*

porary ascendancy during stimulation? The answer to this question is partly indicated by the second of the two considerations to which attention is called, which is as follows: The interior of the living and resting cell, by virtue of the physiological polarization above described, exhibits a very remarkable condition, namely, the presence of a surplus of anions. The influence of the negative charges of these ions must therefore strongly predominate in the interior of the cell, and will accordingly confer on the colloids in this region a certain state of aggregation quite different from that existing in an ordinary non-living colloidal system. The surplus of anions is necessarily only slight, since the electrostatic stress prevents more than a very small proportion of cations from leaving the interior of the cell; still, that it does in fact exist is shown by the electrical contrast between the two sides of the plasma membrane. The interior of the cell being therefore negatively charged, *i. e.*, having a surplus of anions, the large free negative charge carried by this surplus imparts a distinctive consistency to the protoplasmic colloids. Accordingly the protoplasm of living cells is characteristically clear and translucent, indicating a fine state of subdivision of its colloids. The contrast with the condition in dead cells is familiar to all biologists; in brief, protoplasm in dying invariably undergoes coagulation. *Post-mortem* coagulation is, in fact, a widespread and probably universal phenomenon. It is seen in the greatest variety of cells; it is particularly characteristic of muscle where it is usually accompanied by the contraction of *rigor mortis*; it is strikingly shown by eggs with transparent protoplasm, as the starfish egg; and it is a conspicuous phenomenon in the Ctenophore swimming-plate, as I have already described.<sup>10</sup> So widely distributed a phenomenon probably has some general and not specifically chemical explanation; this is to be found (in my opinion) in the loss of the physiological polarization following death. When the polarization is lost, the protoplasmic colloids can no longer maintain the state of fine subdivision characteristic of life and due to the surplus of anions within the cell. Since anions and cations are now present in equal concentration, the colloids adopt an aggregation state different from that previously existing and determined solely by the nature of the electrolytes, independently of any inequality in ionic distribution. The change, since the relative concentration of cations is increased by loss of the polarization,

<sup>10</sup> R. LILLIE: *Loc. cit.*

will evidently be in the direction of coagulation. We must conclude that in the majority of living cells the electrolytes are such as to produce extensive coagulation of the cell colloids in the absence of the physiological polarization. It is this latter condition that gives the anions the ascendancy within the resting cell, — or, in other words, protects the cell colloids from the coagulating influence of the cations. We thus see why, for example, in the *Ctenophore* swimming-plate the structure, clear and translucent in life, becomes opaque white and coarsely granular after death. Similar changes occur — as just mentioned — in many cells, and also in muscle. It is significant that in this last case contraction often accompanies the *post-mortem* coagulation.

These facts and considerations at once indicate a possible explanation of the essential change involved in contraction. During the period of depolarization resulting from the stimulus there is a relative increase in the cations within the cell, producing a change in the direction of coagulation. This, in a tissue consisting of parallel colloidal fibrils, must result in a shortening of these fibrils, *i. e.*, in a contraction. This change reverses itself as soon as the polarization is re-established and the normal condition with intracellular excess of anions is restored.<sup>11</sup> The readiness with which the coagulative change is reversed is, no doubt, due to the brevity of its duration; the quick reversal or relaxation is a direct consequence of the automatic tendency of the surface layer instantly to regain the normal physiological polarization on removal of the disturbing influence. The former condition with surplus of anions, by whose action the coagulative change is reversed, is thus rapidly

<sup>11</sup> Since polarization is essentially a surface phenomenon, it is evident that these changes in ionic concentration will be most marked *at the surface layer of the cell*, immediately within the plasma membrane. Here, therefore, coagulative or other dependent physiological changes will be most pronounced. A strong confirmation of the present theory is hence afforded by the facts (1) that it is precisely in this region that contractile fibrils are first laid down in developing muscle cells of both vertebrates and invertebrates, and (2) that in many muscle cells, particularly in invertebrates, fibrillar differentiation is exhibited throughout life only in the surface layers. For a general description of this structure in invertebrate muscle cells, cf. HEIDENHAIN: "Struktur der kontraktilen Materie," *Ergebnisse der Anatomie und Entwicklungsgeschichte* (MERKEL und BONNET), 1900, x, p. 115. The surface layers of the cell apparently form a critical area for many physiological processes; it seems indeed not unlikely that the main advantage of the cellular form of organization consists in its providing for an enormous extension in the area of the polarized surfaces in the organism.

and automatically regained. The whole event of contraction depends thus on a disturbance of the normal resting equilibrium between the fibrillar colloids and the intracellular ions. This disturbance also alters the chemical equilibrium, as I shall explain shortly; hence the occasion is at the same time furnished for the transformation of chemical energy which, as long recognized, is the ultimate source of the mechanical energy of contraction.

The coagulative change, since it follows directly from the depolarization, can therefore become permanent only under conditions that permanently abolish the physiological permeability. Such conditions, however, necessarily involve the death of the cell; they are actually found in death, as already pointed out; heat rigor is also probably due to alteration of physiological polarization rather than to direct heat coagulation of the colloids. In normal stimulation, on the other hand, the coagulative change can last only as long as the depolarization; and this, as already explained, is, from the conditions of the case, a necessarily evanescent phenomenon and cannot be maintained otherwise than by repeated stimulation, and even then only intermittently. Under abnormal conditions excessive stimulation may lead to a permanent depolarization; but then permanent and irreversible coagulation with consequent death of the contractile elements also results. Thus, as I have already described in detail,<sup>12</sup> the coagulative change in the Ctenophore swimming-plate may show progressive increase during abnormally heightened activity and finally become permanent and irreversible; these effects are seen, for instance, in pure and especially in weakly acidulated solutions of various salts. The explanation is clear from what has just been said, — the effects follow directly from a marked increase in the normal physiological permeability due to the action of the salt on the surface layers of the elements. The transfer of ions thus becomes unusually rapid and is accompanied by unusually rapid contractions; the reversal of permeability — *i. e.*, the restoration of polarization — apparently becomes with each beat progressively more imperfect, and finally normal polarization becomes impossible; under these conditions the colloids coagulate permanently, and the plate is "dead." In more gradual death in sea water similar changes occur; I have already described<sup>12</sup> how a more rapid rhythm, which in the end becomes less intermittent and more difficult to inhibit, precedes the onset of definite mortifier-

<sup>12</sup> R. LILLIE: *Loc. cit.*

ous processes; the dying plates may already show partial coagulation, and a completely coagulated condition is invariably exhibited by dead plates. Analogous phenomena are seen in muscle: twitching may precede the onset of *rigor mortis*, and the appearance of rigor is furthered by previous muscular exertion, — facts both of which indicate the close similarity between the processes of death rigor and of normal contraction. Indeed, as the above considerations sufficiently indicate, the essential difference between the two is that in the dead tissue the depolarization is permanent, while in the living it is necessarily intermittent, the polarized condition being that of equilibrium; hence the coagulation is quickly reversed after stimulation. There are, of course, other differences; in particular the rapid depolarization accompanying stimulation produces far more energetic contractions than those seen during the relatively gradual *post-mortem* alterations. This, however, is completely in agreement with the general rule that sudden changes in the electrolyte content of a colloidal system produce more energetic coagulation than do gradual changes. In both cases, nevertheless, the determining condition of contraction is the coagulative change in the fibrils following the loss of physiological polarization. The resemblance between the chemical changes accompanying the onset of rigor and those of normal contraction has long been recognized. In both instances there is an outburst of carbon dioxide and an increased acidity due to other substances (as lactic acid). The relation of these processes to contraction will be briefly considered later, and the ground of this similarity will then be indicated.

In a complete theory of muscle contraction it would be necessary to account for various other fundamental facts, such, for example, as the relation of sodium salts to irritability. The researches of Overton<sup>13</sup> and Hoeber<sup>14</sup> seem, however, — especially when considered in their relation to each other, — to indicate that the importance of the sodium ion consists essentially in its influence on the permeability of the surface layers of the elements. A certain colloidal consistency in the plasma membrane is requisite for the physiological polarization on which the possibility of stimulation depends; this seems to require the presence of ions in the outer medium; and sodium salts appear especially favorable, although

<sup>13</sup> OVERTON: *Archiv für die gesammte Physiologie*, 1904, cv, p. 176.

<sup>14</sup> HOEBER: *Loc. cit.*



they may be partially replaced by lithium salts and to a lesser degree by caesium or even calcium salts. Hoeber<sup>15</sup> has, in fact, shown that those salts which restore irritability in muscles deprived of this property by immersion in isotonic sugar solutions are precisely those which favor the production of the normal physiological polarization, namely, sodium and lithium salts chiefly, and to some degree alkali earth salts. Hoeber has clearly discussed these and related facts and has pointed out their evident implication, namely, that the stimulation process is essentially conditional on a change in the colloids of the plasma membrane. He shows, further, that these changes are interfered with by anæsthetics, — substances which act directly on the lipoids of the plasma membrane; hence the latter depress or abolish irritability.<sup>16</sup>

Sodium ions can have no specific relation to fibrillar contractility in general, as is shown clearly by the conditions in cilia, where, for example, pure solutions of potassium salts sustain movement far more effectually than do those of sodium salts.<sup>17</sup> The case of the swimming-plate seems more like that of muscle, and here I have found that favorable media always contain large proportions of sodium; media containing sodium, magnesium, and calcium chlorides are the only ones so far found capable of sustaining normal movement for any great length of time in this tissue, which in this respect resembles muscle. Yet contractions are possible in a great variety of salt solutions, though the vibrations are usually abnormally rapid and accompanied by the typical rapid coagulation. The further fact, emphasized in my preceding paper, that contractions accompanied by coagulation occur also in pure solutions of non-electrolytes, shows clearly that external ions are not directly concerned in the contractile process. It seems probable, therefore, that the differences between cilia and muscle relate to certain special features, chiefly peculiarities of surface permeability, rather than to the fundamental conditions of the contractile activity; and that in both cases changes in the concentration or nature of the internally situated ions are directly responsible for the aggregation change conditioning contraction. Since in muscle the electrical change indicates an internal increase in cations during contraction, this

<sup>15</sup> HOEBER: *Archiv für die gesammte Physiologie*, 1905, cvi, p. 599.

<sup>16</sup> Cf. especially *ibid.*, 1907, cxx, pp. 499 *seq.*; also the discussion in *Physikalische Chemie der Zelle*, pp. 271 *seq.*

<sup>17</sup> R. LILLIE: *This journal*, 1906, xvii, p. 89.

fact may be added to the other evidence which, although less direct in the case of this tissue than in that of the swimming-plate, points here also to a coagulative change as the basis of contraction.

The mechanical energy of contraction is clearly transformed chemical energy; and the stages of the transformation must be traced, or at least clearly indicated, in any adequate theory. In the first place, it is evident that the electrolyte to whose ions the polarization is due must be present in higher concentration within than without the cell. This is a necessary condition of polarization. During depolarization the plasma membrane becomes permeable also to the anions, and the entire electrolyte will then diffuse from the cell; activity thus involves a continual loss of the polarizing electrolyte; the latter must therefore continually be produced by the cell, either during or in the intervals of activity. The source of this electrolyte can only be certain chemical reactions within the cell. Again, since the surface energy of the colloidal fibrils is transformed into mechanical energy under the influence of the cations of this electrolyte, the transformed energy must be that of the chemical process yielding this substance. The energy of contraction is thus eventually derived from this process.

As to the nature of the electrolyte or electrolytes concerned in the physiological polarization, — and also, according to the present theory, in the colloidal changes producing contraction, — no very direct evidence is available at present. The cation is not potassium as was formerly supposed for muscle; this has been definitely proved by Hoeber; and it is difficult to assign this rôle to any other cation, unless we except the hydrogen ion. There are, however, many considerations which point in this latter direction. Thus, in the first place acids are known to be produced by oxidative processes in tissues, — carbonic, acetic, formic, and lactic being the chief ones formed in carbohydrate metabolism, which we know to be the main source of muscular energy. Second, the hydrogen ion, as the fastest and most penetrating of all ions, will most readily traverse the surface membrane. Third, this ion has powerful coagulative action on all negative colloids. Fourth, acids are known, even in low concentrations, to be very injurious to contractile processes; weak solutions of acid quickly suppress muscular activity; on the present theory they act by removing polarization. Fifth, no other electrolytes are known to be continually produced in the requisite quantity by the metabolism of contractile tissues, while active muscle pro-

duces carbonic and other acids in considerable quantity.<sup>18</sup> As already mentioned, the electrolyte must be continually lost from the tissue during activity; it must therefore be replaced by the metabolism of the latter. Again, it must be present in relatively low concentration in the external medium. Both of these requirements correspond to the actual conditions of the case.

The fact that stimulation leads to the consumption of energy-yielding material also becomes readily intelligible on our hypothesis. During rest it is to be assumed that an equilibrium exists between the electrolyte (or its anions) within the cell, and the substances by whose interaction it is produced. The diminution in concentration of the electrolyte due to outward diffusion during contraction involves a disturbance of this equilibrium and a progress of the reaction — in accordance with the general principles of chemical equilibrium — in the direction of further production of the electrolyte. The chemical process is thus resumed and continues so long as the reaction product is being withdrawn from the tissue, — that is, so long as stimulation lasts with its accompaniment of increased permeability. The electrolyte — presumably an acid readily convertible to carbonic acid — is thus produced in the interior of the cell, probably in the interior of the contractile fibrillæ or in some other intimate relation to these; the latter then undergo the temporary coagulation producing contraction. When stimulation ceases, the electrolyte at once accumulates, owing to the recovered impermeability of the plasma membrane to its anions; and the reaction ceases. The hydrogen ions penetrating the membrane restore the polarization, and leave the cell with its normal surplus of anions by which the coagulative change is reversed.

The chemical effect of stimulation thus depends on a disturbance of chemical equilibrium due to withdrawal of the reaction products by diffusion (probably accelerated by the contraction itself) through the now freely permeable plasma membrane. So long as the increased permeability remains, the reaction progresses in this direction of oxidative decomposition until the energy-yielding material is depleted; exhaustion then results. The above considerations offer an intelligible point of view from which to explain the otherwise inexplicable fact that a mere change in permeability can oc-

<sup>18</sup> J. LOEB suggested some years ago (this journal, 1902, vi, p. 433) that the ions produced in metabolism might possibly be of importance in the transformation of chemical energy into surface energy in muscle.

casion so remarkable a liberation of energy. It is again to be noted that the *post-mortem* increase of permeability is also associated with a similar liberation of chemical energy, part of which may undergo mechanical transformation, as already seen. Hermann<sup>19</sup> long ago drew attention to the remarkable similarity between the chemical changes accompanying the onset of rigor and those of normal contraction. On the view advocated in the present paper, since the occasion of both is the loss of the normal impermeability and the consequent disturbance of an already existing chemical equilibrium, this similarity is fully in accordance with expectation and requires no special explanation.<sup>20</sup>

#### SUMMARY.

The essential features of the theory presented in the above are as follows:

1. The electromotor properties of the contractile element are explained in accordance with the Ostwald-Bernstein membrane theory. The surface layer of the resting contractile element is permeable to the cations of a certain electrolyte (or electrolytes)

<sup>19</sup> HERMANN: Handbuch, i, pp. 250 *et seq.*, 331 *et seq.*

<sup>20</sup> The above conception of stimulation, as essentially a consequence of increased permeability of the plasma membrane, explains the striking similarity in the action of salt solutions on such outwardly dissimilar processes as glandular secretion and muscular contraction. Pure isotonic solutions of sodium salts induce rhythmical twitching in skeletal muscle, a fact implying increased permeability; the twitching ceases on the addition of a little calcium—*i. e.*, the normal permeability is restored. Similarly, increased permeability of kidney cells, as shown by increased diuresis and glycosuria, follows injection of solutions of sodium salts; and the effect is checked by administration of calcium (J. B. MACCALLUM and M. FISCHER in J. LOEB's laboratory). It may be inferred that in this case also the salts act by altering surface permeability. Such facts suggest the further possibility that many pathological conditions may be directly dependent on alteration of the normal surface permeability of cells. This seems clearly indicated by the conditions in the kidney, where glycosuria and albuminuria, evidence of altered permeability, have long been recognized as the surest signs of derangement. The action of tetanus toxin may similarly be largely due to impairment of the physiological permeability. Restoration of normal permeability, as by administration of calcium,—a procedure long ago advocated by J. LOEB on purely theoretical grounds,—would go far to remove the pathological condition in such cases. It may also be pointed out that changes in ionic permeability imply alteration of the normal ionic content of the cell, and hence alterations in colloidal consistency—phenomena which may well have a pathological aspect.

produced within the element, and impermeable to its anions; thus there arises the typical physiological polarization of the surface layer, with the outer surface positive and the inner negative.

2. This polarization is diminished or removed by any condition that increases the surface permeability and so allows the anions to pass. Such depolarization follows death or injury of the element; it is also temporarily produced by stimulation; any depolarizing agency thus acts as a stimulus. The polarized condition tends automatically to be regained after disturbance; it is therefore the condition of equilibrium in the resting element.

3. In the polarized or resting condition the interior of the element is thus negatively charged, *i. e.*, carries a certain surplus of anions. These confer on the colloids of this region — which are negatively charged — a characteristically fine state of subdivision. Hence the normal clear translucency of “living” protoplasm; this condition, in the contractile element, corresponds to the unstimulated, relaxed, or resting state.

4. During depolarization this surplus of anions diminishes or disappears; the cations in the interior of the element are thus relatively increased, and the colloids thus undergo an aggregation change in the direction of coagulation. Permanent depolarization of a cell, as in death, thus leads to coagulation of the intracellular colloids; thus is explained the typical *post-mortem* coagulation of cells and contractile tissues.

5. A similar but momentary coagulative change in the colloids composing the contractile fibrillæ is the determining condition of normal contraction; in life this change can be only temporary, since the depolarization can be produced only during stimulation, the resting state being one of polarization. The polarized state is thus automatically regained on cessation of the stimulus, and the restored surplus of anions within the contractile element then reverses the coagulative change; hence the return to the resting condition, or relaxation.

6. Reasons are adduced for regarding the polarizing cation as the hydrogen ion. Acids are known to be produced in the contractile element during activity. Also, a necessary condition for the polarization is that the polarizing electrolyte should be continually produced in the element during, or in consequence of, activity, since during stimulation it must be continually lost from the element by diffusion through the then permeable surface-layer.

7. The polarizing electrolyte is thus produced by metabolic changes in the contractile element. The chemical effect of stimulation depends on a disturbance of the chemical equilibrium existing in the resting element between the energy-yielding substance (or substances) and the polarizing electrolyte, which latter is the reaction product of the energy-yielding decomposition. During stimulation the increased permeability allows this product to diffuse freely from the element; hence the reaction progresses in the direction of its further production. The production of this electrolyte is thus the essential condition on which the transformation of the colloidal surface energy in contraction depends. Restoration of impermeability brings the reaction to a rest by the accumulation of the reaction product.

CONTRIBUTIONS TO THE PHYSIOLOGY OF LYMPH. —  
V. THE EXCESS OF CHLORIDES IN LYMPH.

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IN a recent communication by Carlson, Greer, and Becht,<sup>1</sup> it was shown that the osmotic pressure of dog and horse serum collected under light ether anæsthesia is always greater than that of the lymph collected from the neck lymphatics. Their observations also indicated that ether and chloroform anæsthesia increase the osmotic pressure of the serum itself. This point was later verified and extended by Carlson and Luckhardt.<sup>2</sup> Their results render it highly probable that the increase in osmotic concentration of the serum in the anæsthetized animal as compared to that of the normal serum is due solely to the osmotic pressure of the anæsthetic dissolved in the serum.

The results of Carlson, Greer, and Becht on the relative osmotic pressure of serum and neck lymph in dog and horse appear to be contrary to those of Hamburger on the neck lymph and serum of the horse and of Leathes on the thoracic lymph and serum of the dog.<sup>3</sup> According to Hamburger, the osmotic pressure of the neck lymph of the horse is 13 per cent higher than that of the horse serum, while Leathes found that the osmotic concentration of the dog's thoracic lymph exceeded that of the serum by 0.5 to 2 per cent. These results of Hamburger and Leathes are generally regarded as expressing the osmotic relation between lymph and serum in general. There seems to be one possibility of bringing the results of Carlson, Greer, and Becht in line with those of Hamburger and Leathes. The observations of Carlson, Greer, and Becht were all made on lymphs and sera collected under ether or

<sup>1</sup> CARLSON, GREER, and BECHT: This journal, 1907, xix, p. 360.

<sup>2</sup> CARLSON and LUCKHARDT: This journal, 1908, xxi, p. 162.

<sup>3</sup> HAMBURGER: Osmotischer Druck und Ionenlehre, 1904, ii, p. 36; LEATHES Journal of physiology, 1895, xix, p. 1.

chloroform anæsthesia, and it has been shown by Carlson and Luckhardt that ether and chloroform increase the osmotic pressure of the serum in proportion to their concentration in the serum. Now, if during ether and chloroform anæsthesia the serum should hold in solution more of the anæsthetic than does the lymph, the difference in the concentration of the anæsthetic in the two fluids might be sufficient to reverse the osmotic relations obtaining in the normal animal. This possibility is suggested by the old observations of Hammarsten,<sup>4</sup> and Pflüger and Strassburg,<sup>5</sup> that the percentage and tension of CO<sub>2</sub> are less in lymph than in venous blood.

Starling and his school take the position that the higher osmotic pressure of the lymph as compared to that of serum is due to the greater concentration of tissue metabolites in the lymph. This is, as far as we have been able to learn, a mere assumption, as neither Hamburger nor Leathes determined whether their results were due to the greater concentration of organic rather than of inorganic constituents in the lymph. CO<sub>2</sub> may certainly be regarded as a tissue metabolite, but according to Hammarsten and Pflüger the CO<sub>2</sub> tension is less in lymph than even in venous blood. We cannot, therefore, argue *a priori*, from the anatomical relations of the lymphatics to the tissue cells, that the tissue metabolites must be more concentrated in the lymph than in the serum.

Our primary aim in the series of experiments now reported was to obtain serum and lymph under conditions not complicated by anæsthetics, and determine their osmotic concentrations and percentage of inorganic salts, in order to learn whether the difference in osmotic concentration is due to tissue metabolites. In the dog the blood drawn before the anæsthesia is produced does not depress the freezing-point as much as is done by the neck lymph collected during light ether narcosis, while under the conditions of obtaining the blood and serum in the horses there is very little difference in their osmotic concentration. But, to our surprise, the neck lymph of horse and dog contains a greater percentage of inorganic salts and chlorides than does the serum, — a fact which appears irreconcilable with current mechanical theories of lymph formation.

<sup>4</sup> HAMMARSTEN: *Arbeiten aus der physiologischen Anstalt zu Leipzig*, 1871, p. 121.

<sup>5</sup> STRASSBURG: *Archiv für die gesammte Physiologie*, 1872, vii, p. 65.



## I. LITERATURE.

It is commonly assumed that the quantitative and qualitative composition of the inorganic salts in the serum is the same as that in the lymph, as in fact it must be if lymph is a filtrate or a transudate from the blood. But we have been unable to find any extensive work on the quantitative composition of the ash of serum and lymph of the same animal, and it is by such investigations only that the question can be determined. The individual variations in the salt content of the serum are so considerable as to render worthless comparisons between the salt content of the lymph of another animal. To quote some of the figures of the extensive analyses by Bugarsky and Tangl:<sup>6</sup>

Animal.	Number of experiments.	Inorganic salts per 100 c.c. serum.	
		Minimum.	Maximum.
Horse . . . . .	19	0.724	0.870
Swine . . . . .	19	0.800	1.030
Dog . . . . .	12	0.790	0.924

In these experiments the individual variations amount to from 10 to 20 per cent. The same investigators give the individual variations in the salts as figured in gram-molecular concentrations as follows:

Animal.	Total salts, mol. per lit.	Sodium chloride, mol. per lit.
Horse . . . . .	0.224-0.238	0.144-0.184
Ox . . . . .	0.221-0.251	0.169-0.187
Sheep . . . . .	0.251-0.261	0.180-0.214
Swine . . . . .	0.230-0.268	0.134-0.192
Dog . . . . .	0.231-0.254	0.149-0.195
Cat . . . . .	0.256-0.271	0.197-0.219

The extensive analyses by Aberhalden<sup>7</sup> bring out similar individual variations as well as the fairly constant variations in the serum salts of different species of mammals:

<sup>6</sup> BUGARSKY and TANGL: *Archiv für die gesammte Physiologie*, 1898, lxxii, p. 531.

<sup>7</sup> ABERHALDEN: *Zeitschrift für physiologische Chemie*, 1898, xxv, p. 65.

Chlorine per 100 c.c. serum, ox, 0.368; sheep, 0.370; horse, 0.369; rabbit, 0.388; dog, 0.408; cat, 0.417.

In view of these variations in the salt content of the serum of mammals the few analyses of the salt content of mammalian lymph that have so far been made cannot be admitted as proving that the inorganic salts of the serum and the lymph are the same in quantity and quality, because in these lymph analyses parallel determinations were not made on the serum. Most of the analyses of the lymph salts are old and done by inaccurate methods. Hoppe-Seyler<sup>8</sup> cites the following analyses of human lymph:

	Gubler & Guerene: Lymph from fistula in thigh.		Hensen & Dahnhardt: Lymph from fistula in thigh.			Scherer: Lymph fistula spermatic cord.
Inorganic salts per 100 c.c. in gm. }	0.73	0.82	0.83	0.79	1.06	0.83

Schmidt<sup>9</sup> obtained the following figures on the neck lymph and the lymph from the thoracic duct of a colt:

	Neck lymph.		Thoracic lymph.	
Inorganic salts per 100 c.c.	0.72 gm.	0.74 gm.	0.76 gm.	0.75 gm.

Hoppe-Seyler<sup>10</sup> publishes a set of parallel analyses of serum and thoracic lymph from the same animal (dog) with the following figures:

Thoracic lymph, salts per 100 c.c.	= 0.79 gm.
Serum, salts per 100 c.c.	= 0.87 gm.

Nasse<sup>10</sup> determined the sodium chloride content of the thoracic lymph of the dog during hunger, and on carbohydrate and meat diets respectively, finding that the chlorine (NaCl) was diminished on the meat diet as compared to that during hunger and on the carbohydrate diet. The average of his figures for 100 c.c. lymph in NaCl is 0.66 gm. We make no reference to the analyses of pathological transudates and of exudates or of normal pleural and peritoneal fluids, as it is not certain that their formation involves the same mechanisms as that of normal lymph.

It is evident from these lymph and serum analyses that the salt

<sup>8</sup> HOPPE-SEYLER: *Physiologische Chemie*, 1879, iii, p. 591.

<sup>9</sup> Cited from HOPPE-SEYLER: *Ibid.*, p. 592.

<sup>10</sup> HOPPE-SEYLER: *Ibid.*, p. 595.

content of lymph does not differ greatly from that of the serum. The differences that appear fall within the limit of individual variation and probably error of the methods employed.

## II. METHODS.

1. The horses from which the lymph and serum were obtained were specimens condemned to be killed either because of old age or injuries unfitting them for service. The horses were laid low by a blow on the head or a bullet through the brain, one carotid artery immediately excised, and the requisite amount of blood drawn, while the neck lymphatic on one side was at the same time isolated and its contents emptied into a flask by massage of the neck and head region. In this way 10-25 c.c. of lymph, more than enough for analyses, can be obtained from each horse while the heart is still beating, and in some cases even before the respiration stops. The bullet or the blow will at times cut short the respiratory movements at once, in which case the carotid blood becomes decidedly venous before the lymph has been all secured. This blood and lymph are thus not affected by anæsthetics. This method was used in securing the serum and lymph from thirteen horses (Exp. 1-13, Table I).

In Experiment 14 (Table I) the lymph was secured by means of a fistula established under chloroform anæsthesia, the lymph used for the analysis being collected after the recovery from the chloroform. This procedure would undoubtedly have been the best for all the experiments, except for the greater amount of work involved. But we met with the very practical difficulty of restraining the animals after the operation, as we have at present no facilities for suspending or hanging the horses in the temporary quarters serving us as laboratory. In the last three experiments the lymph was collected during light ether anæsthesia and the blood drawn at the end of the experiment under the same degree of anæsthesia.

Attempts were also made to establish temporary neck lymphatic fistulæ in large dogs, but we found it practically impossible to restrain the dogs so as to collect the lymph after the dogs recovered from the anæsthetic. Our analyses of dogs' lymph were therefore made on material collected during light ether anæsthesia. In these experiments the dog's blood was collected from the ear be-

TABLE I.

Horse. Determination of osmotic concentration, total salts and chlorine of serum and lymph from the neck lymphatics. In Experiments 1-13 blood and lymph drawn immediately on the horses being felled with a blow or shot through the brain. Experiment 14, lymph collected from a temporary fistula. Experiments 15-17, lymph collected under light chloroform anæsthesia.

Number of experiment.	Substance.	$\Delta$	Salts per 100 c.c.	Chlorine per 100 c.c.	Excess of chlorine in lymph.
1	Lymph	0.618	0.834	0.344	} 0.041
	Serum	0.605	0.756	0.303	
2	Lymph	0.582	0.814	0.301	} 0.010
	Serum	0.584	0.767	0.292	
3	Lymph	0.625	0.750	0.322	} 0.038
	Serum	0.622	0.720	0.284	
4	Lymph	0.574	0.890	0.366	} 0.075
	Serum	0.593	0.930	0.291	
5	Lymph	0.580	0.900	0.386	} 0.028
	Serum	0.583	0.890	0.358	
6	Lymph	0.607	0.960	0.365	} 0.050
	Serum	0.620	....	0.315	
7	Lymph	0.580	0.940	0.432	} 0.056
	Serum	0.571	0.850	0.376	
8	Lymph	0.604	1.120	0.383	} 0.017
	Serum	0.591	0.990	0.366	
9	Lymph	0.588	1.060	0.386	} 0.059
	Serum	0.597	1.020	0.327	
10	Lymph	0.556	0.980	0.383	} 0.054
	Serum	0.554	0.910	0.329	
11	Lymph	0.583	1.060	0.380	} 0.055
	Serum	0.582	....	0.325	
12	Lymph	0.604	1.040	0.404	} 0.024
	Serum	0.604	0.940	0.380	
13	Lymph	0.607	....	....	} ....
	Serum	0.610	0.990	0.354	
14	Lymph	....	0.790	0.325	} 0.024
	Serum	....	0.800	0.301	
15	Lymph	....	....	0.339	} 0.019
	Serum	....	....	0.320	
16	Lymph	....	....	0.361	} 0.020
	Serum	....	....	0.341	
17	Lymph	0.582	....	0.304	} 0.019
	Serum	0.577	....	0.285	
Average	Lymph	....	....	0.361	} 0.035
	Serum	....	....	0.326	

fore the anæsthesia, a sample was drawn during the experiment, and at the end of the experiment a third sample taken, the anæsthetic being pushed almost to the point of cessation of the respiration. In this way we secured samples of the serum of the same animal without anæsthesia and under varying degrees of anæsthesia, in order to determine whether the inorganic salts play any part in the increased osmotic concentration produced by the anæsthesia.

The neck lymph both of the horse and dog coagulates spontaneously after being drawn. In the case of the lymph used in these analyses the fibrin was always removed. Occasionally the lymph would be contaminated with traces of blood, in which case it was centrifugalized and all erythrocytes removed.

When the fluids were being collected, defibrinated, and centrifugalized, precautions were taken to prevent evaporation and concentration. The serum to be used was invariably freed from corpuscles within an hour or two after being drawn, so as to avoid possible addition of salts from broken-down erythrocytes.

2. *Analytical methods.* — In the parallel series the same quantities of serum and lymph were used — in most cases 5 c.c. In the experiment in which the total ash as well as the chlorine was determined the serum and lymph were evaporated to dryness in porcelain crucibles at  $95^{\circ}$ – $100^{\circ}$  C., then heated up to  $140^{\circ}$  C. for two to four hours, and charred at dull red heat. The charred mass was then extracted repeatedly with boiling water, dried and incinerated, and the washing added, the whole evaporated to dryness and weighed. The chlorides were then determined by Mohr's or Volhard's methods. Both methods gave the same results, but Volhard's is the more convenient, as that renders it unnecessary to make the solution exactly neutral. When only the chlorine was determined, the charred mass was washed with boiling water till the washings became chloride free, the charred mass was then discarded, and the chlorides in the washings determined by the methods just mentioned. In some of the experiments sodium carbonate was added to the sera and lymph previous to evaporation and incineration as a precaution against possible displacement of chlorine by phosphoric and sulphuric acids on incineration. The results were the same as when no alkalis were added.

There are a number of possible sources of error in these procedures. Ammonium salts and possibly potassium chloride may

be lost in the incineration. Potassium chloride does not volatilize at dull red heat, however, and care was taken not to exceed that degree of heat in the incineration. Moreover, the degree and length of heating being the same, there appears to be no reason why the same quantity of volatile salts should not be lost in the serum and the lymph. This would therefore not necessarily be a source of error in our results, as it is the comparative figures of serum and lymph that we are seeking. We made use of small quantities (5 c.c.) of the fluids for the purpose of rendering possible the uniform and rapid charring of the dried residue at dull red heat, and in every case the lymphs were heated for the same time and to the same degree as the serum.

In the second place, it is possible that the phosphoric and sulphuric acids liberated from the proteids may liberate some chlorine, and there being more proteins in serum than in lymph, more chlorine may thus be lost in the serum. That this figures as a source of error in our results is rendered improbable by the fact that the addition of an excess of alkalis before drying and incineration does not change the relative figures. Moreover, the serum, and presumably also the lymph, contains an excess of bases according to the commonly accepted views.

The following check experiments were made to test our results:

Methods.		Chlorides per 100 c.c.	Excess of chlorine in lymph.
I. Horse. Serum and lymph dried and heated to 150° C., extracted with boiling water, filtrate evaporated to dryness and incinerated. Titration by Volhard's method.	Lymph	1. 0.307	0.019
		2. 0.304	
	Serum	1. 0.290	
		2. 0.284	
II. Horse. Serum and lymph the same as in I. Dried with excess of Na <sub>2</sub> CO <sub>3</sub> , and charred at dull red heat. Volhard's method.	Lymph	1. 0.302	0.019
		2. 0.302	
		3. 0.298	
		4. 0.298	
		5. 0.302	
	Serum	1. 0.284	
2. 0.280			
3. 0.280			
III. Horse. Serum and lymph by the Neumann method. <sup>11</sup>	Lymph	0.306	0.043
	Serum	0.263	

On the whole, our results by the Neumann method ran lower than with the straight Volhard and Mohr methods, possibly because

<sup>11</sup> NEUMANN: *Zeitschrift für physiologische Chemie*, 1902, xxxvii, p. 115. Suggested to us by our colleague, Dr. KOCH.

of the difficulty of getting all the chlorine distilled over as hydrochloric acid. But the relative results were the same, the lymph showing a greater percentage of chlorine than the serum. The check experiments, I and II, balance up very close. In neither of these two experiments is there any possibility of loss of chlorine through displacement by phosphoric or sulphuric acids.

### III. RESULTS.

1. In every one of our seventeen experiments on horses (Table I), as well as in our check experiments, *the lymph shows a higher percentage of chlorine than does the serum*. This difference in favor of the lymph averages about 10 per cent both in the horse and in the dog. In the experiment in which the total salts were also determined a similar difference appears in favor of the lymph, except in Experiments 4 and 14. In Experiment 14 this difference is so slight as to fall within the limits of possible errors in the titration. In none of our dog experiments were the total salts determined.

2. *There is no increase in the chlorides of the dog's serum drawn under light and deep ether anæsthesia as compared with the chlorides of the serum before the administration of the anæsthetic*. This is brought out by the figures in Table II. The parallel freezing-point determinations show the same variations as those found by Carlson and Luckhardt. The osmotic pressure of the normal serum is less than that of the serum drawn under light anæsthesia, and the deeper the anæsthesia the greater the depression of the freezing-point. If there is any increase in the chlorides of the serum under ether anæsthesia, it is too slight to be measured by our present methods. This is additional evidence in support of the conclusion reached by Carlson and Luckhardt, that the increase in osmotic concentration of the serum during ether and chloroform anæsthesia is due to the anæsthetics themselves dissolved in the serum.

It is therefore evident that for the purpose of quantitative comparisons of the salts in lymph and serum of the same animal the material may be collected under ordinary ether and chloroform anæsthesia without introducing any sources of error.

3. In all our dog experiments *the osmotic concentration of the serum before administration of the anæsthetic is less than that of the neck lymph collected during light anæsthesia, while the relations*

are reversed when the comparisons are made between this lymph and the serum drawn simultaneously. We are not in position to

TABLE II.

Dog. Determination of osmotic concentration and total chlorine in parallel series of serum and lymph from the neck lymphatics, all the lymphs collected under light ether anæsthesia.

Number of experiment.	Substance.	$\Delta$	Chlorine per 100 c.c.	Excess of chlorine in lymph.
1	1. Lymph right neck lymphatic (1)	....	0.431	0.035
	2. Lymph right neck lymphatic (2)	0.675	0.420	
	3. Lymph right neck lymphatic (3)	0.671	0.432	
	4. Lymph left neck lymphatic (1)	0.666	0.431	
	5. Lymph left neck lymphatic (2)	....	0.429	
	6. Serum before anæsthesia	0.623	0.397	
	7. Serum during anæsthesia	0.697	0.390	
2	1. Lymph right neck lymphatic (1)	0.650	0.447	0.043
	2. Lymph right neck lymphatic (2)		0.460	
	3. Lymph left neck lymphatic (1)		0.453	
	4. Lymph left neck lymphatic (2)		0.455	
	5. Serum before anæsthesia	0.626	....	
	6. Serum during anæsthesia	0.675	0.411	
	7. Serum, very deep anæsthesia	0.683	0.411	
3	1. Lymph right neck lymphatic	....	0.443	0.017
	2. Lymph left neck lymphatic	....	0.429	
	3. Serum during anæsthesia	....	0.419	
4	1. Lymph right neck lymphatic (1)	0.638	0.444	0.031
	2. Lymph right neck lymphatic (2)		0.436	
	3. Lymph left neck lymphatic (1)		0.439	
	4. Lymph left neck lymphatic (2)		0.446	
	5. Serum before anæsthesia	0.625	0.417	
	6. Serum during anæsthesia	0.650	0.409	
	7. Serum, very deep anæsthesia	0.672	0.419	
5	1. Lymph right neck lymphatic	0.682	0.361	0.026
	2. Lymph left neck lymphatic		0.365	
	3. Lymph thoracic duct	0.719	0.368	
	4. Serum before anæsthesia	0.680	0.339	
	5. Serum during anæsthesia	0.704	0.338	
	6. Serum, very deep anæsthesia	0.746	0.339	
Average	1. Lymph		0.426	0.030
	2. Serum		0.396	

say whether or not the anæsthetic increases the osmotic pressure of the lymph similar to that of the serum, but if it does perceptibly increase that of the lymph, this increase is certainly much less than in the serum. To actually determine this point it will be necessary to make parallel measurements of the percentage of the anæsthetic



in serum and lymph. This indicates that the osmotic pressure of the neck lymph of the dog is higher than that of the serum, but ether and chloroform anæsthesia reverses this normal osmotic relation.

Under the conditions of collecting the lymph and serum in the horses there is not much difference in their osmotic concentration (Table I). The difference is sometimes in favor of the lymph, at other times in favor of the serum. Series 1 to 14 are not complicated by anæsthetics. It has been shown by Carlson, Greer, and Becht that under conditions of chloroform anæsthesia the difference is in favor of the serum. The present series of experiments do not therefore seem to support Hamburger's conclusion that the neck lymph of the horse has a higher (13 per cent) osmotic concentration than the serum.

Bottazzi has shown that the freezing-point of serum is lowered by the addition of  $\text{CO}_2$ , and that the depression is the greater the more  $\text{CO}_2$  in solution in the serum. Under the conditions of collecting the serum and lymph in Experiments 1-14 the blood was usually very venous. The lymphs were in all probability less saturated with  $\text{CO}_2$ , in the first place, because most of the lymph collected consisted of the lymph in the large lymph vessels that had been formed in the tissues while the animal was breathing normally. And, according to Hammarsten and Pflüger, the  $\text{CO}_2$  tension in the lymph is even normally less than in venous blood.

It is therefore possible that in our present experiments on horses the  $\text{CO}_2$  of the blood increased the osmotic concentration of the serum to such an extent that the normal excess of osmotic concentration of the lymph over the serum is obscured. This is suggested by the fact that Hamburger's result on the horse is apparently duplicated by our present experiments on the neck lymph and serum of the dog, using a method different from that of Hamburger. It does not seem probable that there should be any such fundamental difference between the dog and the horse, and we are therefore inclined to agree with Hamburger that under normal conditions the osmotic concentration of the neck lymph of the horse is greater than that of the blood. But we cannot argue from this fact that osmosis is an important factor in lymph formation, because the osmotic relation between the lymph and the serum is reversed by ether and chloroform anæsthesia, and yet the lymph continues to flow unchecked.

4. Bogarsky and Tangl estimated that  $\frac{3}{4}$  of the osmotic concentration of the serum is due to the electrolytes, and  $\frac{1}{4}$  to organic constituents and undissociated molecules. The same observers conclude that the organic moiety of the osmotic concentration is subject to greater individual variations than the inorganic salts. The excess of osmotic concentration in the lymph is attributed by Starling and his school to the excess of the organic constituents (tissue metabolites). There is no evidence for this view. In fact, one of the end products of tissue activity ( $\text{CO}_2$ ) has been shown to be under higher tension in venous blood than in lymph. The subject requires further investigation rather than further discussion; but according to our results *the excess of chlorides in the lymph is more than sufficient to account for the difference in osmotic concentration.*

5. *The excess of chlorides in the lymph seems to us to render the filtration and transudation theories of lymph formation untenable.* The reverse relation might have been reconciled with the mechanical theory, because it is conceivable that some of the chlorides might be held in combination with the proteins, and thus be osmotically inactive. And as there is a much greater percentage of proteins in the serum than in the neck lymph, there might then be more chloride in the serum than would be possible on the filtration theory in case all the chlorides were free. But we can see no way of harmonizing the excess of salts and chlorides in the lymph with any of the mechanical theories of lymph formation, unless we accept the newer views of the nature of the processes of osmosis advanced by Kahlenberg,<sup>12</sup> Battelli and Stephani,<sup>13</sup> and others. According to their work, the freezing-point does not constitute a measure of the osmotic pressure of a solution. It seems to us desirable that these views should be more firmly established by the chemists and the physicists before we attempt to make use of them in explaining physiological phenomena, but if these theories shall prove to be well founded, all that part of physiology based on the old and commonly accepted theory of the nature of osmosis must be entirely recast and the work to a great extent repeated. It need not be pointed out that our results are not adverse to a purely mechanical explanation on Kahlenberg's theory.

<sup>12</sup> KAHLENBERG : Journal of physical chemistry, 1906, x, p. 141.

<sup>13</sup> BATTELLI and STEPHANI : Physikalisches Zeitschrift, 1906, vii, p. 190.

Our purpose in this report is merely to record the facts observed. We have no explanation of how this excess of chlorides in lymph over that in the serum comes about or what use it serves in the body economy. The explanation is probably to be sought in the relation of the lymph to the tissues rather than in the relation of the lymph to the blood.

CONTRIBUTIONS TO THE PHYSIOLOGY OF LYMPH. —  
VI. THE LYMPHAGOGUE ACTION OF LYMPH.

By A. J. CARLSON, J. R. GREER, AND F. C. BECHT.

[From the Hull Physiological Laboratory of the University of Chicago.]

IN our previous report on the mechanism effecting the transfer of water from the blood to the tissue spaces in the active salivary glands, we concluded that it could not be an osmotic mechanism, as the freezing-point of the lymph from the active salivary gland (parotid, horse) may be higher than that of the serum of the same animal.<sup>1</sup> It will be recalled that these parotid lymphs as well as the sera were collected under chloroform anæsthesia. Since the publication of that report it has been shown by Carlson and Luckhardt, and by Carlson, Greer, and Luckhardt<sup>2</sup> that the osmotic pressure of the serum is increased by ether and chloroform in direct proportion to the depth of the anæsthesia, and that in all probability this increase in osmotic concentration is due solely to the anæsthetic in solution in the serum. There is at least no increase in the percentage of chlorides of the serum under anæsthesia as compared to normal serum. The results of Carlson, Greer, and Luckhardt also indicate that during ether and chloroform anæsthesia more of the anæsthetics are held in an osmotically active form in the serum than in the neck lymph. For example, the osmotic concentration of the neck lymph of the dog is greater than that of the normal serum, but less than that of the serum collected during light and deep anæsthesia. Now, inasmuch as the increased osmotic concentration of the serum in anæsthesia is due mainly, if not solely, to the osmotic pressure of the anæsthetics themselves, the conclusion seems inevitable that less ether and chloroform is held in osmotically active forms in the lymph than in the serum.

<sup>1</sup> CARLSON, GREER, and BECHT : This journal, 1907, xix, p. 360.

<sup>2</sup> CARLSON and LUCKHARDT : This journal, 1908, xxi, p. 162; CARLSON, GREER, and LUCKHARDT : *Ibid.*, 1908, xxii, p. 91.

In view of these facts it is possible that under normal conditions the osmotic concentration of the lymph coming from the active salivary glands is higher than that of the serum, our previous results to the contrary notwithstanding, as the higher osmotic pressure of the serum in our experiments may have been due to the excess of chloroform in the serum. The experiments do show, however, that under light chloroform narcosis water leaves the blood in the active salivary glands against an osmotic pressure that may be considerable. We can see no way of harmonizing this fact with any osmotic mechanism capable of effecting this water transfer. The fact that under normal conditions the osmotic concentration of the salivary lymph is in all probability greater than that of the blood proves nothing in favor of an osmotic mechanism, as the water transfer proceeds just as rapidly when these osmotic relations between the lymph and the serum are reversed by anæsthetics.

The discovery that lymph contains more chlorides than the serum offers an explanation of the lower freezing-point of the serum as compared with the lymph, while it seems to us to render untenable all theories of lymph formation based solely on filtration, diffusion, and osmosis as these processes are commonly understood to-day. The lymph is constantly being passed into the blood; hence the chlorides must constantly be passing into the lymph against diffusion and osmosis. The greater concentration of chlorides in the lymph cannot come from the tissues except indirectly, for in that case the tissues would soon be rendered chloride free. Our previous conclusion that "the mechanism effecting the water transfer from the blood to the lymph in the active salivary glands is either an 'hormone' or secretory nerves to the capillaries" is therefore strengthened rather than weakened by the later results.

The present report deals with some experiments designed to test our hormone hypothesis. While the hypothesis was stated with special reference to the salivary glands, it is obviously applicable to the processes of lymph formation in all the organs of the body, and our present work has assumed this wider scope, especially because the measurement of the rate of lymph formation in small organs like the salivary glands is so difficult that small variations in the rate easily fall within the limits of experimental errors.

## I. THE LITERATURE.

The previous observations that bear most closely on our problem are those D'Errico carried out in Bottazzi's laboratory.<sup>3</sup> The starting-point of D'Errico's experiments was the observation of Kaufmann<sup>4</sup> and Hamburger<sup>5</sup> that the lymph flow from the neck lymphatics of the horse in locomotion is greater than when the horse stands still, although the head remains quiet under both conditions, and, as Hamburger has shown, the blood pressure in the capillaries of the head region is less in the walking horse than in the horse standing still. The obvious explanation of this fact is that the active muscles of the limbs and trunk produce some substance which passes into the blood and increases the formation of the lymph in other (quiescent) parts of the body. To test this hypothesis D'Errico studied the effect on the lymph flow from the thoracic duct (dog) of injecting intravenously defibrinated blood from dogs fatigued by electrical stimulation. He found that the blood and serum from fatigued dogs greatly augmented the lymph flow, while similar quantities of blood drawn from the same animals prior to being fatigued had no effect on the lymph flow. To obviate increased blood pressure by the injections, a quantity of blood equal to that to be injected was drawn from the animal prior to the injection. This lymphagogue action of defibrinated blood and serum from the fatigued animals of the same species appears immediately after injection and may persist for an hour. D'Errico interprets these results in accordance with Asher's theory, the increased lymph flow being secondary to organ activity, heightened by the substances present in the blood after fatigue. These substances, then, act primarily to increase organ activity which leads to the liberation of osmotically active metabolites, their discharge into the lymph, and the withdrawing of the water from the blood capillaries by osmosis. In short, the mechanism of this lymph formation is, in the last analysis, osmotic pressure.

Asher bases his theory of lymph formation on the above hypothesis,<sup>6</sup> but it may also be reconciled with the filtration hypothesis,

<sup>3</sup> D'ERRICO : *Archives internationales de physiologie*, 1905, iii, p. 168.

<sup>4</sup> KAUFMANN : *Archives de physiologie*, 1892, iv, p. 279.

<sup>5</sup> HAMBURGER : *Archiv für Physiologie*, 1895, p. 363 ; 1897, p. 132.

<sup>6</sup> ASHER and BARBÉRA : *Zeitschrift für Biologie*, 1898, xxxvi, p. 154.

inasmuch as increased organ activity is normally associated with increased blood supply and consequent increased capillary pressure. Starling considers this osmotic mechanism only, according to his latest statement,<sup>7</sup> and that is also the position of Barcroft, Bainbridge, and other investigators. Our hypothesis that one of the normal mechanisms of lymph formation is a hormone produced in the normal activity of the tissues is therefore new only in so far as it postulates that these hormones act by augmenting the normal "secretory" activity, and not by osmosis, or pathologically, by injuring and thus increasing the permeability of the capillary endothelium.

## II. EXPERIMENTAL METHODS.

If our hypothetical hormones have a direct action in the production of lymph, it would seem probable that some of these hormones are present in the lymph, at least before the lymph has sojourned in the lymph glands. On this hypothesis we proceeded to test the lymphagogue action of the lymph itself. Large-sized dogs under light but uniform ether anæsthesia were used. Canulas were inserted in the thoracic duct and in one or both neck lymphatics, and the rate of the lymph flow in drops recorded on the kymograph in the manner described in our previous communication.<sup>8</sup> The lower jaw of the dog was massaged by the mechanical device described in the paper just referred to. In the present series of experiments the apparatus was run by an electric motor so as to give absolute uniformity in rate and intensity of movements. This uniformity is not attained, however, unless the degree of anæsthesia remains constant as variations in the tonus of the jaw and neck muscles introduce variable factors of resistance.

The dogs used were starved for twenty-four to thirty-six hours before the experiment so that the thoracic lymph might not have exceptional composition, owing to the state of digestion or the nature of the food.

In most cases the lymph injected was that collected from the animal under observation. A period of from fifteen to thirty minutes was allowed to elapse between the beginning of the recording

<sup>7</sup> STARLING: Recent advances in the physiology of digestion, 1906, p. 60.

<sup>8</sup> CARLSON, GREER, and BECHT: This journal, 1907, xix, p. 360.

of the lymph flow and the first injection of the lymph, and in case of the thoracic lymph this period would yield more than the necessary quantity for injection. When lymph from other dogs was used, it was never more than four hours old, and during the interval between drawing it from one dog and injecting it into another it was kept in the ice box, so as not to introduce error from bacteria and bacterial products.

The lymph was defibrinated, warmed to body temperature, and injected very gradually into the femoral vein. In four preliminary experiments the arterial blood pressure was not recorded, but in the last eight experiments the pressure in the femoral artery was recorded throughout the experiments. As the injection of the dog's own lymph is only returning the quantity of the fluid lost by bleeding from the lymphatics, such injection ought not to increase its volume of fluid in the blood vessels and thus increase the arterial or capillary pressure. In several cases, however, before injecting 20 or 30 c.c. of the animal's own lymph or lymph from another dog, we drew the same quantity of blood from the femoral artery.

By taking the average rate of flow for a considerable period the error from occasional clotting, variation in the degree of anæsthesia, and consequent rate of intensity of respiration, etc., are clearly obviated.

### III. RESULTS.

1. *Lymph flow from the thoracic duct.* — In all of our twelve experiments *the intravenous injection of the animal's own thoracic and neck lymph, or the same lymphs from another dog, is followed by an increased flow of lymph from the thoracic duct.* The percentage of increase is variable, as will be seen from inspection of Table I. On the whole, the greater the quantity of lymph injected the greater the lymphagogue action, but the condition of the animal as well as the character of the lymph is also an important factor, so that these quantitative relations are not always evident.

This augmentation of the lymph flow is equally marked on injection of the animal's own lymph as on injection of the lymph from another dog. Moreover, the neck lymph appears to be as efficient as the lymph from the thoracic duct.

The augmentation of the thoracic lymph may appear within a minute or two after the beginning of the injection. But this condition is rather exceptional. Frequently the increase is not in evi-



dence until five to eight minutes after the injection. The augmented lymph flow persists from one-half to one and one-half hours.

The only factor studied accurately was the rate of flow. D'Errico found that after the injection of the blood from fatigued dogs the thoracic lymph became mixed with blood. It is not unusual to find the lymph flowing from the thoracic duct mixed with a trace of blood, especially in dogs that fight violently on being anæsthetized. In our earlier experiments we found occasionally that this would occur towards the end of prolonged experiments. In the present series we observed it in only two cases after the injection of the lymph. Hence it is not a necessary result of the lymph injection.

2. *The lymph flow from the neck lymphatics.* — Our results from the flow from the neck lymphatics are so far unsatisfactory. There are some practical difficulties in recording the rate of flow from the thoracic duct and the neck ducts at the same time, as all workers in this field will concede. In three of our experiments (Table I) there is a slight increase in the neck lymph flow following the lymph injection. In the last experiment (Table I, 8) there is the usual gradual diminution in the rate that always occurs under normal condition. The records in Experiments 4 to 7 could not be used because of errors from clotting, etc. The rate of lymph flow from the neck lymphatics in a dog under ether anæsthesia and resting on its back is relatively slow, even under our system of moving the lower jaw. The rate of flow can be augmented by massage and stroking of the neck by the hand, but this is inadmissible in these experiments, as it is impossible to keep this type of massage uniform. The rate varies greatly in different animals, but there is under normal conditions a gradual decrease with the duration of anæsthesia and the experiment. Because of this normal gradual diminution in the rate of flow an actual slight augmentation may follow the lymph injection without bringing the rate even up to that prior to the injection.

In short, our results indicate that these lymph injections produce a slight augmentation of the lymph flow from the neck lymphatics just as in the case of the thoracic lymph flow, but more work is required to prove the point definitely.

3. *The effect on blood pressure.* — The slow injection of 20 to 40 c.c. of defibrinated neck and thoracic lymph from the same animal or from another dog has no effect on the arterial pressure. Rapid injection may cause a temporary rise or a slight temporary

fall, but the pressure soon returns to normal. This is the case whether or not prior to the injection a quantity of blood equal to the lymph injected was withdrawn from the animal. The aug-

TABLE I.

The effect of intravenous injection of lymph on the rate of lymph formation. Dog. Light ether anæsthesia. The rate of the lymph flow is recorded in number of drops per minute.

No. of exp.	Time in min.	Rate of flow before injection.		Lymph injection.	Time in min.	Rate of flow after injection.	
		Neck lymph.	Thoracic lymph.			Neck lymph.	Thoracic lymph.
I	25	1.70	15.50	25 c.c. of own thoracic lymph.	30	2.00	17.00
II	15	0.87	6.00	20 c.c. neck lymph from another dog.	40	1.10	8.00
III	19	1.33	5.00	20 c.c. thoracic lymph from another dog.	40	1.77	7.70
	40	1.77	7.70	30 c.c. of own thoracic lymph.	40	1.56	13.75
	..	....	....	....	30	1.20	15.00
IV	22	....	34.00	30 c.c. of dog's own thoracic lymph.	31	....	44.00
	31	....	44.00	40 c.c. of own thoracic lymph.	14	....	46.00
V	13	....	10.20	25 c.c. of own thoracic lymph.	43	....	11.20
	43	....	11.20	20 c.c. of own neck lymph.	23	....	18.00
VI	16	....	24.00	25 c.c. of own thoracic lymph.	41	....	28.20
	41	....	28.20	40 c.c. of own thoracic lymph.	20	....	33.00
VII	10	....	6.90	20 c.c. of neck lymph of another dog.	30	....	7.90
	30	....	7.90	25 c.c. of own thoracic lymph.	42	....	11.80
VIII	46	3.20	15.50	35 c.c. of own thoracic lymph.	53	2.71	19.90
	53	2.71	19.90	40 c.c. of own thoracic lymph.	15	2.11	25.00

mented lymph flow cannot therefore be due to or related to increased arterial pressure. A separate series of measurements is necessary to determine whether similar lymph injections increase the pressure in the portal system.

In some of our experiments the injection of the defibrinated lymph produces typical Traube-Hering waves on the pulse tracing from the femoral artery lasting from ten to twenty minutes. This is

apparently the same phenomenon as is described by Asher and Barbéra following reinjection of neck lymph secured by passive massage of the neck. According to Asher and Barbéra the substance in the lymph producing these effects on the vasomotor centre is produced in the tissues and rendered innocuous or ineffective in the lymph glands. Because of the massage of the head and neck region in collecting the lymph for injection Asher and Barbéra think that the lymph did not remain long enough in the lymph glands for this change to be effected.

In our experiments we observe the Traube-Hering phenomenon one time after the injection of neck lymph and three times after the injection of lymph from the thoracic duct. The lymph from the thoracic duct used in our injection was secured without massage, and exactly in the conditions that it normally empties into the blood, as there are no lymph glands near the junction of the thoracic duct with the vein. And under the conditions of our experiments the massage of the head and neck region was certainly much less than in the normal chewing and swallowing. The head lymph was therefore not forced through the lymph glands at a greater rate than normally. Nevertheless, both the neck lymph and the thoracic lymph on defibrination and injection may produce Traube-Hering curves. It is therefore evident that the substance responsible for this action is present in the lymph after leaving the lymph glands, contrary to Asher's conclusion.

The condition of the animal into which the lymph is injected appears also to be an important factor. For example, the injection of the animal's own defibrinated lymph from the thoracic duct may produce a temporary Traube-Hering phenomenon, while a second injection of another sample of the same lymph thirty minutes later has no such effect. It is, of course, possible that in such a case the second sample of lymph collected may contain less of this toxic substance, although collected from the same animal under precisely the same conditions.

4. *Are we permitted to conclude from these results that in the normal animal the lymph carries to the blood some substance which, when carried back to the capillaries and the tissues, increases the lymph production?* — Our experimental conditions differ from those in the normal animal in the following points:

(1) The lymph is formed and collected under anæsthesia, and is introduced into the blood of the animal under anæsthesia.

(2) The lymph is defibrinated, and probably contains in considerable quantity the products of broken down leucocytes.

(3) Even on relatively slow injection of the lymph the lymph will reach the blood in greater quantity in the same units of time than is the case in the normal animal.

(4) The animal into which the lymph is injected is being deprived of its own lymph for variable periods before the injection.

These facts are, of course, obvious, and while it is certain that under the condition of our experiments lymph has a lymphagogue action, we frankly admit that this does not constitute a demonstration that lymph has the same action under normal conditions. It seems to us, however, that our results render it probable that such is the case.

There appears to be no way of a closer approximation of the conditions of the experiments to those of the normal animal. The lymph may be collected from temporary fistulæ without anæsthesia, but in this case the injection will have to be made into another animal. And there is no way of detecting small variations in the lymph flow from the neck lymphatics and the thoracic duct except with the animal quiescent under some anæsthetic.

5. *The mechanisms involved in the lymphagogue action of lymph.*—The more recent work on the structure and development of the lymphatic system has not received adequate attention from physiologists, despite the fact that this work renders untenable the old view that the lymphatics are in open communication with the tissue spaces, or, in fact, only a continuation of the tissue spaces. On this older theory of the structure of the lymphatics the only factors that need to be taken into consideration in the formation of lymph are the (1) walls of the blood capillary, and (2) tissue cells. This is the position taken by nearly all writers on lymph and lymph formation. This position is untenable.

The researches of Budge,<sup>9</sup> Ranvier,<sup>10</sup> Florence Sabin,<sup>11</sup> McCallum,<sup>12</sup> and others have shown conclusively that the lymphatic system is a diverticulum from the veins and grows into the organs by processes of budding. The lymphatic system, therefore, is a closed system lined throughout with endothelium. The closed lymph capil-

<sup>9</sup> BUDGE: *Archiv für Anatomie*, 1880, p. 320; 1887, p. 59.

<sup>10</sup> RANVIER: *Archives d'anatomie microscopique*, 1897, i, p. 137.

<sup>11</sup> SABIN: *American journal of anatomy*, 1901, i, p. 367; 1905, iv, p. 355.

<sup>12</sup> MCCALLUM: *Archiv für Anatomie*, 1902, p. 273.

laries dip into the tissue spaces, but are not continuous with them. American anatomists have had no little share in the establishment of this thesis. In the symposium on the structure and development of the lymphatic system at the annual meeting of the Association of American Anatomists in Chicago, January 1-3 of this year, this position was supported by such an array of facts as to render it almost impregnable. It is therefore time that the physiologists take cognizance of this work and remodel their conceptions accordingly. A closed lymphatic system implies that we must distinguish between two kinds of lymph in general, namely, (1) the fluid within the lymphatic vessels, and (2) the fluid in the tissue spaces or the tissue lymph. It scarcely needs to be remarked that we know nothing directly of the physiology of the fluid in the tissue spaces. This new position compels us, furthermore, to recognize a third factor in lymph formation, namely, the endothelial walls of the lymphatic acini. The three factors to be reckoned with in lymph formation are (1) the walls of the blood capillaries; (2) the walls of the lymph capillaries; (3) the tissue cells.

Our hypothetical lymph hormone, formed during the activity of the tissue cells, may therefore augment lymph formation by acting on (1) the walls of the blood capillaries; (2) the walls of the lymph capillaries; (3) the walls of both systems of capillaries. Condition 1 results in formation of tissue lymph, condition 2 in the formation of lymphatic lymph. Assuming for the present that these hormones are the active mechanism, condition 1 is certainly predominant in the salivary glands, and possibly in all glands where activity involves the elaboration and elimination of great quantities of fluids.

The lymph hormones must in the first instance come in contact with and act on the side of blood capillary cells facing the tissue spaces. If we reason from the analogy of the fate of the better-known hormones in the body, it is highly probable that the lymph hormones are dissipated or changed in the cells on which they act. Hence under normal conditions probably only a few of the hormones pass in their active form into the lumen of the blood capillaries or into the tissue lymph. In other words, the main action of the lymph hormones is local. Some of the hormones probably reach the lymphatic lymph in their active condition. Their numbers may be further diminished or increased in the lymph glands. On reaching the blood stream the hormones may again be brought

in contact with the walls of the blood capillaries and exert this action in the formation of tissue lymph. It is conceivable that this activity of the blood capillaries involves the formation of hormones, which in turn act on the walls of the lymph capillaries in a way to augment the formation of lymphatic lymph.

Our results are, however, capable of a different interpretation. None of the lymph hormones may get into the lymphatic lymph or directly into the lumen of the blood capillaries. They may all be changed in passing through the lymph capillary and blood capillary wall. The lymphagogue action of lymph obtained by us would on these conditions be due to some substance or substances which after passing through the blood capillary walls act on the tissue cells in a way to increase the rate of formation of the lymph hormones. A study of the structures of the lymphagogue substances in the lymph might aid in deciding between these two possible mechanisms.

The evidence that the mechanism by which the tissue cells effect lymph formation is not osmosis is, in brief, the following:

1. Lymph continues to be produced under conditions where the osmotic pressure of the serum is much greater than that of the lymph (ether and chloroform anæsthesia).
2. The greater osmotic concentration of the lymph over that of the serum which normally exists, at least in the case of lymph from certain organs of the body, is probably due to the excess of chlorides in the lymph rather than to the excess of tissue metabolites.

These statements apply to the old and generally accepted theory of the nature of osmotic pressure. The facts are not irreconcilable to Kahlenberg's theory of the nature of osmotic pressure, as we have pointed out in a previous communication.

There remains the possibility that our hypothetical lymph-forming hormones may produce their effects by injury to or the initiation of pathological processes in the walls of the blood capillaries. This is the well-known interpretation of Starling and his school of the mechanism of the action of Heidenhain's lymphagogue of the first class. While conceding the possibility of this mechanism in the case of our tissue lymph hormones, we think most physiologists will agree with us that such a mechanism of action of substances formed in the normal life processes of the organisms is highly improbable. Now, since the osmotic pressure theory is contrary to actually demonstrated facts, and as the injury hypothesis is highly im-

probable on general grounds, there remain only the secretory mechanisms above suggested and discussed as indicating the direction in which the solution of the problem of lymph formation is to be solved.

The fact that, in many instances at least, the activity of the salivary gland and of the pancreas does not increase the flow of lymph from these glands seems to indicate that the processes of formation of lymphatic lymph and tissue lymph do not always run parallel. The one process may be augmented, while the other remains unaffected. In organs whose activity does not involve the elimination of great quantities of fluid to the exterior (muscle, nervous tissue, glands of internal secretion), activity is probably always associated with increased formation of lymphatic lymph, while in organs like the salivary glands, the pancreas, and possibly the kidneys, the formation of tissue lymph must increase with activity, while the formation of lymphatic lymph may not. It is possible that the breakdown of the mechanism effecting the co-ordination of the rate of formation of the lymphatic lymph and the tissue lymph is an essential factor in edema.

## A QUANTITATIVE STUDY OF FARADIC STIMULATION.—II. THE CALIBRATION OF THE INDUCTORIUM FOR BREAK SHOCKS.

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THE introductory paper of this series was devoted to a preliminary discussion of the variable physical factors whose values must be known in quantitative estimations of faradic stimuli. The first of these factors to be considered was the inductorium, and it was shown that this requires two independent calibrations, — one for break shocks, the other for make shocks. The Kronecker graduations were shown not to be available in coils as commonly used, being based upon measurements made with coils from which the iron cores had been removed.

**Physiological method of comparing stimuli.** — In preparing the calibration to be presented herein, it has been constantly kept in view that although faradic stimuli are in themselves essentially physical, and measurable by physical methods, for use as physiological agencies their values must be correlated to physiological standards. In other words, calibrations based upon physical determinations must be shown to express physiological relationships with accuracy if they are to satisfy the needs of physiologists. Since this requirement calls for knowledge of the physiological values of stimuli, which can be known only from the responses of living tissues to them, and since we have at present no way in which to judge accurately the relative values of different responses, it is necessary to use an indirect method of comparing stimuli. The plan has been adopted of using for this purpose a constant response, compensating for variations in one factor involved in the stimulus by changing others, and observing the amount of change required to restore the constant response. The constant response selected for this work is an adaptation of the one originally sug-



gested by v. Fleischl,<sup>1</sup> namely, the minimal contraction of an isolated uncurarized frog's gastrocnemius. It was found by experiment that this muscle suspended in a moist chamber at constant temperature usually maintains a uniform irritability for several hours after isolation.

In detail the method employed was as follows: The freshly isolated gastrocnemius was suspended by its attached femur in a moist chamber, and its lower end connected by a small copper wire to a muscle lever whose effective weight was about 10 gm.; the muscle was not afterloaded. The lever had a magnification of about ten, and its point pressed lightly upon a smoked drum. The minimal contraction could be detected without difficulty, since the whole apparatus was made rigid enough for the slightest movement of the muscle to show itself at the end of the lever. Connection between the muscle and the terminals of the secondary coil was by means of two platinum needles soldered to fine copper wires leading from the secondary terminals. These needles were thrust directly through the muscle tissue, — one about 5 mm. below its origin, the other the same distance above the distal tendon, both in the same vertical plane. By this method of connecting the muscle variations in the secondary resistance aside from those in the tissue itself were avoided. At least half an hour was allowed to elapse after the muscle was hung in position before stimulation was begun; in order that summation might not enter, the shortest interval allowed between successive stimuli was thirty seconds; to avoid fatigue the strength of stimulus used was always kept as near minimal as possible. The results of repeated experiments show that under these conditions a high degree of constancy is usually maintained during the interval, about three hours, required for a single experiment. It is, of course, necessary that each experiment be complete in itself, since no means has suggested itself for obtaining a response which shall remain constant through a period of successive days.

#### BREAK SHOCKS.

Helmholtz<sup>2</sup> appears to have been the first to study in detail break induction shocks. He established the principles which are

<sup>1</sup> v. FLEISCHL: Sitzungsberichte der Wiener Academie, Mathematische-physische Classe, lxxii, -3, p. 42.

<sup>2</sup> HELMHOLTZ: POGGENDORF'S Annalen der Physik und Chemie, 1851, lxxxiii, p. 505.

still accepted as to their formation and course. His work was chiefly from the physical standpoint, although he gave attention also to the physiological aspect of the problem. Recently Fleming<sup>3</sup> has given a clear and concise discussion of break induction shocks, his presentation agreeing in every essential particular with the earlier one of Helmholtz. The following preliminary statement is, in the main, condensed from Fleming's discussion.

**The course of break induced currents.** — The current induced in a secondary coil by the breaking of the primary current may be represented graphically by such a curve as is given in Fig. 1, beginning at zero, increasing rapidly to a maximum, and then falling more slowly away to zero. If the break at the primary were absolutely instantaneous, the initial rise would not take place, and the secondary current would begin with its maximum value. Since, however, there is always, even under most favorable conditions, a certain amount of sparking at the contacts, there is never an instantaneous break, and the initial rise is constantly present. Helmholtz<sup>4</sup> demonstrated, with the aid of an ingenious apparatus, that the physiological effect of a break induced current is all exerted by that part embraced within the ascending limb of the curve. By breaking the secondary current at various points in its course he found that the physiological effect was just as great when the current was broken at the moment of reaching its maximum intensity as when it was allowed to run its entire course. From the standpoint of the physiologist, therefore, that part of the curve represented by the area  $ACB$  of the diagram is the only part that need be taken into account. The factor of this area which is of importance in quantitative estimations of the physiological effects of secondary currents is the ordinate  $CB$ , drawn from the base line to the summit of the curve, since, according to Fleming,<sup>5</sup> the physiological effect varies directly as the maximum intensity. That author states that the steepness of the curve is also of importance; that is, of two currents of equal maximum intensity the one which reaches the maximum more quickly will have the greater physiological effect. Neither the theoretical nor the experimental basis for either of these statements is given by Fleming.

The abscissa  $AB$  represents the time occupied by the spark,

<sup>3</sup> FLEMING: The alternate current transformer, London, 1892, i, pp. 184 *et seq.*

<sup>4</sup> HELMHOLTZ: *Loc. cit.*

<sup>5</sup> FLEMING: *Loc. cit.*, p. 180.

Helmholtz<sup>6</sup> having shown that the induced current reaches its maximum intensity at the instant the spark ceases to pass. In a properly constructed apparatus  $AB$  will be constant. The value of the ordinate  $CB$  is approximately equal to  $\frac{MI}{L}$ , in which  $M$  is

the mutual inductance between primary and secondary,  $I$  the intensity of the current through the primary, and  $L$  the self-inductance of the secondary. If the break were instantaneous, making  $AB$  equal to zero,  $CB$  would equal the expression given above; it falls below that value more and more as  $AB$  increases, but so long as  $AB$  is constant the re-

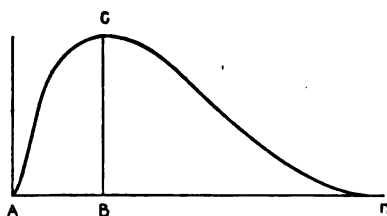


FIGURE 1. — Curve illustrating the course of a break induced current — After Fleming.

lation between the true value of  $CB$  and the value  $\frac{MI}{L}$  which it approximates will not vary.

We have, then, in this expression a physical basis for the measurement of break induction shocks. The remaining paragraphs of this paper are devoted to a description of the methods for determining the values of the factors involved, and to demonstrating that the expression  $\frac{MI}{L}$  is a true measure of the physiological intensity of break shocks so long as the time occupied in breaking the primary circuit remains constant.

#### DETERMINATION OF THE FACTORS IN THE EXPRESSION $\frac{MI}{L}$ .

Of the three factors which make up the expression  $\frac{MI}{L}$ , one,  $I$ , the intensity of the primary current, is an easily measured electrical quantity, and is best determined directly by means of an ammeter in the primary circuit. The other two,  $M$  and  $L$ , are functions of the construction of the inductorium, either by itself or as modified by the relative positions of the primary and secondary coils.  $M$ , the mutual inductance between the primary and secondary coils, varies with changes in the position of the secondary relative to the primary, but is fixed for each position. It can therefore be deter-

<sup>6</sup> HELMHOLTZ: *Loc. cit.*

mined once for each position of the secondary coil, and the values thus obtained used in all future calculations.

$L$ , the self-inductance of the secondary coil, is a function of the construction of the secondary coil, and is therefore constant, for a given inductorium, so long as it remains unaffected by extraneous influences. When the secondary coil is brought up over the iron core of the primary, its self-inductance is, however, thereby markedly increased, so that the constant value of  $L$  holds only in that part of the field beyond the influence of the iron core. It was found by experiment that the value of  $L$  begins to increase appreciably at the point where the secondary coil just fails to clear the primary.

**Method of determining mutual inductance.** — For determining the values of  $M$  advantage is taken of the fact that this factor appears in the expression for the integral effect of the induced current. This integral effect is represented in the diagram (Fig. 1) by the entire area  $A B C D$ ; its expression is  $\frac{MI}{R}$ , in which  $M$  and  $I$  have the same meanings as hitherto, and  $R$  equals the resistance in the secondary circuit. The integral effect can be measured by means of the ballistic galvanometer.

The various methods of determining the values of  $M$  by means of the ballistic galvanometer are given in detail in works on electrical measurements. In brief, the one used in this research was as follows: The secondary of the induction coil under examination was connected in series with a moving-coil ballistic galvanometer of the d'Arsonval wall type and with the secondary of a standard induction coil, the latter apparatus being so constructed that the mutual inductance between its primary and secondary coils could be computed from the construction of the apparatus and the current through the primary. The special features of its construction were that the primary was a solenoid of one-layer thickness, very evenly wound, and several times longer than the secondary, so that the lines of force through the latter, which was at the middle of the primary, were practically straight.

The secondary of the inductorium whose values of  $M$  were desired was set successively at points 1 cm. apart, beginning at zero. At each point the galvanometer deflection caused by breaking a primary current of known intensity was determined. Since each galvanometer deflection represents a certain integral effect, no matter how produced, and since the integral effect affords means of computing  $M$ , it was only necessary to determine the intensity of current which had to be broken in the primary of the standard coil to produce these same deflections

to have at hand all the data required for calculating the values sought. The formula used for computing  $M$  was developed in the following manner: The expression for the integral effect is, as stated above,  $\frac{MI}{R}$ .

Let this represent the galvanometer deflection caused by breaking a current of intensity  $I$  in the primary of the coil whose values of  $M$  are desired. Let the expression  $\frac{M'S}{R}$  represent the same galvanometer deflection caused by breaking a current of intensity  $S$  through the primary of the standard coil. Equating these, we have  $\frac{MI}{R} = \frac{M'S}{R}$ .

The method of connecting the secondaries was, as stated previously, purposely such that the value of  $R$  is constant throughout. It therefore disappears from the equation and we have  $MI = M'S$ . The value of  $M'$  is computed from the construction of the standard coil according to the formula  $M' = 4\pi nNAS$ , in which  $n$  equals the number of turns in the primary coil per centimetre of length,  $N$  the total number of turns in the secondary coil,  $A$  the area of the cross section of the primary, and  $S$  the current through the primary in electromagnetic units. Since this current is measured in amperes, it is necessary in practice to call  $S$  the intensity of the primary current in amperes and divide the expression by 10 to reduce to electromagnetic units. The formula for  $M'$  then becomes  $\frac{4\pi nNAS}{10}$ . The value  $\frac{4\pi nNA}{10}$  is constant for any given standard coil. In the present instance it equalled 32182.7, so that the formula for the mutual inductance became  $M = \frac{32182.7 \cdot S}{I}$ .

Table I gives the values of  $M$  as determined for the inductorium to be known in this series of papers as coil  $B$ .

If  $\frac{MI}{L}$  is a true expression for the physiological effect of break shocks, it is evident that in the part of the field where  $L$  is constant the product  $MI$  must also be constant so long as it represents a uniform stimulus, no matter how the value of  $M$  may be varied by shifting the secondary coil. As a matter of fact, experiment shows that when allowance is made for a source of variation to be discussed presently,  $MI$  does remain constant for a constant stimulus over the entire field of the inductorium except that part of it directly over the iron core of the primary. This is the region in which, as shown in a former paragraph, the value of  $L$  increases.

The primary coil of the inductorium used in this work was about 14 cm. long, and the value of  $L$  became constant when the secondary was 14 cm. from the zero position.

**Correction for the magnetization of the core.**—The source of variation referred to above for which allowance must be made under certain circumstances is the magnetization of the iron core. This,

TABLE I.  
Values of  $M$  at different positions of the secondary coil. — Coil B.

Position of secondary in centimetres.	Value of $M$ .	Position of secondary in centimetres.	Value of $M$ .	Position of secondary in centimetres.	Value of $M$ .
0	16,120,000	14	960,000	28	49,000
1	15,400,000	15	650,000	29	43,000
2	14,530,000	16	460,000	30	38,000
3	13,400,000	17	345,000	31	33,800
4	12,150,000	18	265,000	32	30,300
5	10,800,000	19	212,000	33	27,300
6	9,420,000	20	166,000	34	24,800
7	8,000,000	21	138,000	35	22,800
8	6,560,000	22	116,000	36	20,900
9	5,270,000	23	98,000	37	19,100
10	4,100,000	24	84,000	38	17,400
11	3,050,000	25	73,000	39	15,800
12	2,190,000	26	64,000	40	14,300
13	1,466,000	27	56,000		

as was stated in the former paper (p. 72), becomes appreciable when primary currents above a certain intensity (in this work 0.1 ampere) are used. The method of allowing for the magnetization of the core is by the introduction of a correction term derived in the manner described below.

The correction term for the magnetization of the core can be obtained without difficulty by the use of the ballistic galvanometer, since the deflections of that instrument are affected by it. Inspection of the formula  $MI = 32182.7.S$  shows that so long as  $M$  remains constant,  $I$ , the current through the primary of the coil under examination, must vary directly as  $S$ , the current through the primary of the standard coil. It was found by experiment that this relationship holds in the coils used in this work for values of  $I$  up to 0.1 ampere, but above that point the value of  $S$  is always larger than the equation calls for. This variation due to the magnetization of the core is not, however, very difficult to correct, because, as repeated experiment has shown, the ratio between the actual values of  $I$  and those computed from the

values of  $S$  depends upon a factor which is constant for any given iron core over its entire field.

Table II gives the average results of seven experiments performed with coil  $B$  illustrating this point. The decimal part of each ratio given

TABLE II.

Value of $I$ observed in amperes.	Value of $S$ observed.	Value of $I$ computed in amperes.	Ratio computed value of $I$ to its observed value.	Observed value of $I$ divided by decimal part of ratio.
0.01	0.005	0.01	1.0	....
0.05	0.025	0.05	1.0	....
0.10	0.05	0.10	1.0	....
0.20	0.1044	0.2088	1.044	4.55
0.30	0.1597	0.3194	1.065	4.60
0.40	0.2180	0.4360	1.090	4.44
0.50	0.2782	0.5564	1.113	4.43
0.60	0.3396	0.6792	1.132	4.55

in column four represents the proportional part of itself by which the corresponding observed value of  $I$  must be increased to give the computed value of  $I$ . As column five shows, these decimals bear a constant relationship to the values of  $I$  which they affect, in coil  $B$  equal to  $1 \div 4.5$ . This relationship may be stated as the equation  $x = 4.5 y$ , in which  $x$  equals the observed value of  $I$  and  $y$  the proportional part of itself by which this value must be increased to give the computed value of  $I$ . This equation holds over the entire field of the inductorium and for all primary currents between the beginning of appreciable magnetization and complete magnetic saturation. In the coils employed in these measurements, as already stated, the former point was reached at about 0.1 ampere; the latter was not obtained with a primary current of 0.75 ampere, which was as much as was used in these experiments on account of the sparking at the contacts.

**Demonstration that strengths of break shocks vary over the outer part of the field as the product of the mutual inductance by the primary currents.**—By the use of the equation  $x = 4.5 y$  as described in the preceding paragraph, the proper correction factors for the magnetization of the iron core can be readily introduced where required. The method is now complete for demonstrating

by means of constant responses that break shocks vary as  $MI$  in the part of the field where the value of  $L$  is constant. In Table III the results of six experiments are set down. In performing these

TABLE III.

Pos. of sec. cm.	Val. of $M$ .	EXP. OF JAN. 3, 1907.			EXP. OF APR. 17, 1907.			EXP. OF APR. 18, 1907.		
		Val. of $I$ in amp.	Corr. factor.	Val. of $MI$ .	Val. of $I$ in amp.	Corr. factor.	Val. of $MI$ .	Val. of $I$ in amp.	Corr. factor.	Val. of $MI$ .
14	960,000	....	....	....	0.008	....	7,680	....	....	....
16	460,000	....	....	....	0.016	....	7,360	0.0203	....	9,340
20	166,000	0.040	....	6,640	0.048	....	7,950	0.0573	....	9,510
24	84,000	0.080	....	6,720	....	....	....	0.113	1.024	9,720
28	49,000	0.130	1.028	6,550	0.152	1.034	7,700	....	....	....
32	30,300	0.205	1.046	6,500	0.23	1.051	7,330	0.290	1.064	9,350
36	20,900	0.297	1.066	6,620	0.337	1.075	7,570	0.415	1.092	9,470
40	14,300	0.426	1.094	6,660	....	....	....	0.570	1.127	9,190
		EXP. OF APR. 19, 1907.			EXP. OF MAY 17, 1907.			EXP. OF JAN. 16, 1908.		
14	960,000	0.0160	....	15,360	0.00695	....	6,670	....	....	....
16	460,000	0.0335	....	15,410	0.01450	....	6,670	0.0285	....	13,100
20	166,000	0.0890	....	14,770	0.04020	....	6,670	0.0788	....	13,080
24	84,000	0.170	1.038	14,810	....	....	....	0.15	1.033	13,000
28	49,000	....	....	....	....	....	....	0.255	1.057	13,220
32	30,300	0.445	1.099	14,810	....	....	....	....	....	....
36	20,900	....	....	....	....	....	....	....	....	....
40	14,300	....	....	....	....	....	....	....	....	....

experiments great care was taken to keep all conditions not being directly investigated as uniform as possible. Analysis of the table shows clearly that the variations from a constant value of  $MI$  for any given experiment are well within the limits of experimental error. In Table IIIa is given the average percentage variation



from the mean value of  $MI$  for each experiment, and also the maximum percentage variation from the mean.

When it is considered that errors might enter in reading the ammeter, in determining the point of minimal contraction, and

TABLE III a.

Date.	Average percentage variation.	Maximum percentage variation.	Date.	Average percentage variation.	Maximum percentage variation.
Jan. 3, 1907	0.9	1.7	April 19, 1907	1.87	2.47
April 17, 1907	2.33	4.3	May 17, 1907	0.0	0.0
April 18, 1907	1.45	3.0	Jan. 16, 1908	0.45	0.91

in setting the secondary coil, and also that changes in the irritability of the tissue are liable to occur at any time, these variations are seen to be no greater than are to be anticipated in work of this kind.

**Method of determining the self-inductance of the secondary coil.**

— By definition the coefficient of self-inductance of any circuit is numerically equal to the number of lines of force which thread the circuit when it bears a current whose intensity is unity in electromagnetic measure (Daniell). Now, the number of lines of force which thread a coil is proportional to the product of the mean cross section of the coil by the number of turns of wire of which it is composed. Experiment has shown that this product, which is proportional to the self-inductance of the coil, answers completely for the purposes of the present calibration as a measure of the value of  $L$ , and it has therefore been adopted in this work as representing that value.

In Table IV is given the experimental demonstration that the product of the mean cross section of any secondary coil by the number of turns of wire of which it is composed may be substituted for  $L$  in the expression  $\frac{MI}{L}$ , and that the expression so obtained is constant for a constant stimulus, whether the stimulus is produced by one inductorium or another.

For obtaining this proof five inductorias were employed. The data from which the values of  $L$  were obtained are as follows:

Coil.	Mean cross-section.	Turns in secondary.	Product. "value of $L$ ."	Length of secondary.	Remarks.
	sq. cm.			cm.	
B	17.6	10,350	182,000	13	A large Hasler inductorium with Kroecker graduations.
C	22	4,830	98,500	7.4	An old inductorium of ordinary form.
D	22	5,145	113,000	6.5	The secondary coil from an old inductorium used with the primary coil of inductorium B.
E	8.05	5,000	40,000	6.5	An inductorium of the Harvard Apparatus Company. This coil is the property of the psychological department and was kindly calibrated for me by Dr. Yerkes.
F	15.4	3,000	46,000	13.5	The secondary was wound in this laboratory. The primary coil of inductorium B was used as the primary coil of this apparatus.

In connection with the determination of the value of  $L$  it should be noted that a high degree of accuracy in measuring the mean cross section of the secondary coil is essential to the success of the determination. This is a measurement which is made with difficulty on the completed coil. It would therefore enhance the values of inductoria which are designed for quantitative work if their makers would include, in addition to the usual statements of the number of turns in the secondary coils, accurate statements of their mean cross sections.

For the sake of bringing within convenient limits the final units by which the strengths of stimuli are to be expressed the values of  $L$  used in Table IV and all subsequent tables are the values determined as above divided by 100. When comparisons such as are given in Table IV of the stimuli produced by different inductoria are made by the method used in this work, care must be taken that the direction of flow of the induced current through the tissue used as an indicator is the same throughout each comparison. The stimulating effect of a break induced current passed through an uncurarized gastrocnemius according to the method described at the beginning of this paper is much greater when the anode is next to the tendo achilles than when the current passes in the reverse direction.

TABLE IV.

Demonstration that the method indicated for determining the value of  $L$  makes the expression  $\frac{MI}{L}$  constant for constant break stimuli when different inductoria are compared. Each horizontal line of the table represents a uniform strength of stimulus.

Date.	Coil.	Val. of $M$ .	Val. $\frac{M}{L}$ .	Val. $I$ in amp.	Val. $\frac{MI}{L}$ .	Coil.	Val. of $M$ .	Val. $\frac{M}{L}$ .	Val. $I$ in amp.	Val. $\frac{MI}{L}$ .
1908. Jan. 15	B	460,000	250.0	0.012	3.00	E	920,000	2300.0	0.00135	3.10
	"	166,000	91.2	0.0313	2.86	"	410,000	1020.0	0.00313	3.19
	"	84,000	46.0	0.0615	2.83	"	110,000	275.0	0.0112	3.08
	"	49,000	27.0	0.115	3.20 <sup>1</sup>	"	45,000	112.0	0.031	3.47
	"	166,000	91.2	0.0365	3.33	C	69,000	70.0	0.0455	3.18
	"	84,000	46.0	0.0725	3.33	"	40,000	41.3	0.078	3.22
	"	49,000	27.0	0.118	3.27 <sup>1</sup>	"	26,000	26.4	0.13	3.52 <sup>1</sup>
Jan. 16	"	84,000	91.2	0.15	7.14 <sup>1</sup>	E	110,000	275.0	0.026	7.15
	"	49,000	27.0	0.255	7.28 <sup>1</sup>	"	45,000	112.0	0.062	6.95
	"	460,000	250.0	0.021	5.25	C	135,000	137.0	0.042	5.75
	"	49,000	27.0	0.19	5.34 <sup>1</sup>	"	26,000	26.4	0.2	5.50 <sup>1</sup>
Jan. 23	"	166,000	91.2	0.0545	4.96	"	69,000	70.0	0.0685	4.80
	"	84,000	46.0	0.101	4.75 <sup>1</sup>	"	40,000	41.3	0.12	5.08 <sup>1</sup>
Jan. 30	"	460,000	250.0	0.047	11.75	F	114,000	248.0	0.0475	11.80
Jan. 31	"	460,000	250.0	0.026	6.50	D	310,000	274.0	0.0224	6.14
	"	460,000	250.0	0.0283	7.08	F	114,000	248.0	0.0278	6.95
	D	310,000	274.0	0.0223	6.12	"	114,000	248.0	0.0243	6.10
	"	310,000	274.0	0.0203	5.56	"	114,000	248.0	0.0224	5.60

<sup>1</sup> The correction factor for the magnetization of the core has been introduced.

Determination of the values of  $\frac{M}{L}$  in that part of the field where the value of  $L$  is not constant. — In a previous paragraph it was stated that when the secondary coil is brought up over the iron core of the primary its self-inductance is thereby increased. This increase is greater the further the secondary coil is brought over the core.

The amount of it for any given position of the secondary coil, although constant, cannot readily be computed nor measured directly. Experiment has shown, however,

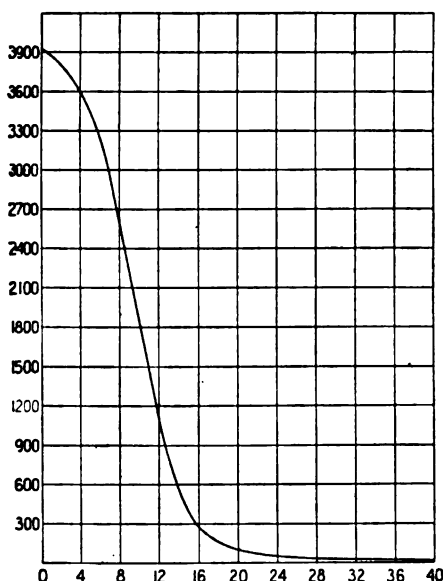


FIGURE 2.—The calibration curve for Coil B. Ordinates represent positions of the secondary coil in centimetres. Abscissas represent values of  $\frac{M}{L}$ .

physiological method to determine the value of the expression  $\frac{M}{L}$  for each point

within the region of increased self-inductance of the secondary coil, and thereby to satisfy the requirements of the present scheme of calibration. The method used depends upon the fact already demonstrated that in the outer part of the field the expression  $\frac{MI}{L}$  is constant for a

constant stimulus. The value of this constant was determined for the minimal contraction of a certain muscle, and then the values of  $I$  were found which produced the same response in the same tissue with the secondary coil

at different points over the iron core. The constant value of  $\frac{MI}{L}$  divided by the value of  $I$ , for any position of the secondary coil gives a number which is fixed for that position and which represents the value of  $\frac{M}{L}$  for it. By taking the average results of a large number of experiments these numbers were established with sufficient accuracy. In Table V are given the values of  $\frac{M}{L}$  established by this method for coil B together with six experiments demonstrating the essential accuracy of the method.

The complete calibration for coil B as worked out for the even scale positions is presented in Table VI. In the table are presented also the results of two experiments which are introduced for the

TABLE V.

The values of  $\frac{M}{L}$  for the region of Coil B about the iron core of the primary coil.  
Experimental demonstration of their essential correctness.

Pos. of sec.	Val. of $\frac{M}{L}$	EXP. OF DEC. 24, 1906.		EXP. OF JAN. 26, 1907.		EXP. OF APR. 19, 1907.	
		Val. of $I$ in amp.	Val. of $\frac{MI}{L}$	Val. of $I$ in amp.	Val. of $\frac{MI}{L}$	Val. of $I$ in amp.	Val. of $\frac{MI}{L}$
0	3,920	0.00053	2.08	0.00137	5.37	0.00217	8.50
1	3,900	....	....	0.00138	5.38	....	....
2	3,820	....	....	0.00146	5.58	....	....
3	3,720	....	....	0.00149	5.54	....	....
4	3,600	0.00060	2.16	0.00151	5.44	0.00223	8.03
6	3,200	....	....	0.00166	5.32	....	....
8	2,600	0.00080	2.08	0.00194	5.04	0.00325	8.45
10	1,880	....	....	0.00272	5.11	....	....
11	1,500	....	....	0.00336	5.04	....	....
12	1,100	0.00197	2.17	0.00461	5.07	0.00740	8.14
16	250	0.0093	2.32	0.02100	5.24	0.0335	8.38
		EXP. OF MAY 13, 1907.		EXP. OF MAY 17, 1907.		EXP. OF MAY 18, 1907.	
		Val. of $I$ in amp.	Val. of $\frac{MI}{L}$	Val. of $I$ in amp.	Val. of $\frac{MI}{L}$	Val. of $I$ in amp.	Val. of $\frac{MI}{L}$
0	3,920	0.00155	6.08	0.00093	3.65	0.00091	3.57
1	3,900	....	....	....	....	....	....
2	3,820	....	....	....	....	....	....
3	3,720	....	....	....	....	....	....
4	3,600	0.00160	5.76	0.00099	3.56	0.00094	3.38
6	3,200	....	....	....	....	....	....
8	2,600	0.00230	5.98	0.00131	3.41	0.00134	3.48
10	1,880	....	....	....	....	....	....
11	1,500	....	....	....	....	....	....
12	1,100	0.00570	6.27	0.00315	3.47	0.00310	3.41
16	250	....	....	0.01450	3.62	0.01475	3.69

TABLE VI.

Complete calibration for Coil B, even scale divisions. Two experiments illustrating the substantial accuracy of the calibration.

Pos. of sec.	Value of $\frac{M}{L}$ .	EXP. OF JAN. 3. 1907.		EXP. OF APRIL 18, 1907.	
		Value of $I$ in amp.	Value of $\frac{MI}{L}$ .	Value of $I$ in amp.	Value of $\frac{MI}{L}$ .
0	3920.0	0.0009	3.53	0.00126	4.94
1	3900.0	....	....	....	....
2	3820.0	....	....	....	....
3	3720.0	....	....	....	....
4	3600.0	0.00097	3.49	0.00138	4.97
5	3420.0	....	....	....	....
6	3200.0	....	....	....	....
7	2940.0	....	....	....	....
8	2600.0	0.00133	3.46	0.00184	4.78
9	2240.0	....	....	....	....
10	1880.0	....	....	....	....
11	1500.0	....	....	....	....
12	1100.0	0.0033	3.63	0.00456	5.02
13	800.0	....	....	....	....
14	530.0	....	....	0.01035	5.48
15	360.0	....	....	....	....
16	250.0	0.0154	3.85	0.0203	5.08
17	190.0	....	....	....	....
18	145.0	....	....	....	....
19	116.0	....	....	....	....
20	91.0	0.04	3.64	0.0573	5.21
21	76.0	....	....	....	....
22	64.0	....	....	....	....
23	54.0	....	....	....	....
24	46.0	0.08	3.68	0.113	5.33 <sup>1</sup>
25	40.0	....	....	....	....
26	35.0	....	....	....	....
27	31.0	....	....	....	....
28	27.0	0.13	3.61 <sup>1</sup>	0.167	4.68 <sup>1</sup>
29	23.5	....	....	....	....
30	21.0	....	....	....	....
31	18.5	....	....	....	....
32	16.5	0.205	3.53 <sup>1</sup>	0.29	5.10 <sup>1</sup>
33	15.0	....	....	....	....
34	13.5	....	....	....	....
35	12.0	....	....	....	....
36	11.5	0.297	3.64 <sup>1</sup>	0.415	5.20 <sup>1</sup>
37	10.5	....	....	....	....
38	9.5	....	....	....	....
39	8.7	....	....	....	....
40	7.8	0.426	3.64 <sup>1</sup>	0.57	5.02 <sup>1</sup>

<sup>1</sup> The correction for the magnetization of the iron core of the primary has been introduced.

sake of presenting in compact form evidence corroborative of that given in sections in various parts of the paper to the effect that this calibration, based in the main on physical determinations, affords a true comparison of the physiological intensities of break induction shocks.

TABLE VII.

Verification of the calibration of Coil *B* for intermediate positions of the secondary coil.

Position of secondary cm.	Value of $\frac{M}{L}$	EXP. OF APRIL 6, 1907.		EXP. OF APRIL 13, 1907.	
		Value of <i>I</i> in ampere.	Val. of $\frac{MI}{L}$	Value of <i>I</i> in ampere.	Val. of $\frac{MI}{L}$
2.0	3820.0	0.0021	8.02	0.00082	3.13
5.35	3300.0	0.0025	8.24	0.00090	2.97
8.2	2520.0	0.0032	8.06	0.00113	2.85
11.28	1390.0	0.0059	8.20	0.00203	2.82
12.45	970.0	0.0088	8.54	0.00300	2.91
14.0	530.0	0.0158	8.37	0.00560	2.97
16.2	238.0	0.0355	8.44	0.01240	2.95
18.44	132.0	0.0650	8.58	0.02100	2.77
21.2	73.0	0.1150	8.62 <sup>1</sup>	0.03900	2.85
25.2	39.0	0.2150	8.78 <sup>1</sup>	0.07600	2.96
29.1	23.3	....	....	0.12000	2.87 <sup>1</sup>

<sup>1</sup> The correction factor for the magnetization of the iron core of the primary has been introduced.

**Calibration of intermediate positions on the scale.**—The values of  $\frac{M}{L}$  for points on the scale intermediate between those determined experimentally can conveniently be obtained by constructing a curve in which these values are plotted against their corresponding scale divisions. The curve for coil *B* is given in Fig. 2. For practical usefulness the curve must be constructed on a large scale. The substantial accuracy of the intermediate values obtained by this method is shown in Table VII. The secondary positions selected in these experiments were indicated by Kronecker graduations ranging from 12,000 to 30.

The method of calibration outlined herein is submitted with the belief that it will prove to be an advance step toward increased facility in the quantitative use of induction shocks. In subsequent papers the calibration of the inductorium for make shocks and the variable factors remaining to be considered will be discussed.

#### SUMMARY.

1. The physiological intensities of break induction shocks are proportional, other factors remaining constant, to the expression  $\frac{MI}{L}$ , in which  $M$  is the mutual inductance between the primary and secondary coils,  $I$  the intensity of the primary current, and  $L$  the self-inductance of the secondary coil.

2. The mutual inductance between the primary and secondary coils varies with changes in the position of the secondary coil relative to the primary coil, but is fixed for each position. Its value can be determined by a comparatively simple physical method.

3. The self-inductance of the secondary coil is proportional to the product of the number of turns of wire in the coil by its mean cross section. It is therefore fixed for any given inductorium so long as it remains unaffected by extraneous influences.

4. When the secondary coil is brought up over the iron core of the primary coil, its self-inductance is thereby markedly increased, necessitating a special determination of the values of  $\frac{M}{L}$  for this region. These values are established by a simple physiological method.

5. Primary currents whose intensity exceeds one tenth ampere cause appreciable magnetization of the iron core of the primary coil. This in turn affects the physiological intensities of break shocks and must be taken into account by the introduction of a correction factor. The degree of magnetization of the core depends upon its construction, and also bears a fixed relation to the intensity of the primary current. The formula for computing the required correction factor can be determined for any inductorium by a simple physical method.



## STUDIES IN HEART MUSCLE.—THE REFRACTORY PERIOD AND THE PERIOD OF VARYING IRRITABILITY.

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IN the present paper it is desired to describe the result of a series of experiments in which the excitability of heart muscle during systole, diastole, and the so-called post-diastolic pause was studied. The main object of the investigation was to determine, if possible, whether the refractory period is variable, and, if variable, what are the conditions necessary to alter its duration. At the same time considerable attention was paid to the variation in irritability of the muscle during the phases following its period of contraction. With this end in view experiments, in which induction shocks of varying intensity were used, were performed on different parts of the heart treated with (1) sodium-potassium-calcium solution, (2) varying percentages of calcium chloride and potassium chloride in a 0.7 per cent solution of sodium chloride, and (3) varying temperatures of the saline bath.

In regard to the method used, a general description has already been given.<sup>1</sup>

<sup>1</sup> "The effect of chloral hydrate upon the properties of heart muscle," this journal, August, 1906, pp. 484-485. A few details that have not hitherto been described may be given here. The stimulating coil was a large Petzolt coil, the secondary of which had 3000 windings of No. 36 wire, the primary having 125 windings of No. 14 wire. The scale was in millimetres, thus making it possible to vary the distance of the secondary from 0 to 420 mm. During the first part of the investigation the primary was connected with one Edison-Lalande cell, type Q; later Columbia dry cells were used. As a rule, each cell when tested showed a voltage of from 1 to 1½. A series of seventeen cells was used in part of the work, and when tested showed a voltage of 19-20. Zinc-oxide storage cells, prepared by Dr. Eyster of this laboratory, proved most satisfactory. These showed a voltage of from 2 to 2½ each, and a 50-ampere hour current. Two of these connected in series could be used for long periods of time with slight change in voltage.

The ventricular strips were in general prepared by starting the cut near the base of the ventricle on one side and continuing the incision parallel with the apical edge until the base of the ventricle of the opposite side was reached. It was usually divided into right and left halves by making a vertical incision in the region of the phrenum. A two and a half millimetre glass tube was bent L-shaped, into one end of which a fine platinum wire was fused, thus allowing the included end of the wire to form a good contact with the mercury with which the tube was subsequently filled. The free end of the wire was bent into a hook, forming the fixed point to which the lower end of the strip was fastened, the upper end being fastened to another small platinum hook suspended vertically from the free end of the writing lever by means of a silk thread. The upper hook, being also connected with a short piece of tinsel or a piece of copper wire, formed one electrode, and the lower hook formed the other. In part of the experiments the strip was protected from the drying influence of air by means of a glass cap fitted snugly over the vessel of saline bath. A piece of thin rubber dam covered the cap, within the centre of which a glass tube was fitted, thus enabling the lever thread to move freely.

**I. Sodium-potassium-calcium solution** <sup>2</sup> (with especial reference to varying the intensity of stimuli). — It was found that strips cut while in Ringer solution <sup>2</sup> and suspended in a moist chamber do not at first respond to induction shocks even of considerable intensity ( $S\ 120\ P_2$ ).<sup>3</sup> For thirty minutes or longer they remain inexcitable. First responses are usually in the form of partial contraction that are less than one millimetre in extent. The excitability is low, and stimuli of moderate strength ( $S\ 150$  to  $120\ P_2$ ) are effective only after two or more have passed through the muscle.<sup>4</sup> Soon after the appearance of partial contractions the excitability gradually increases, especially when the strip is subjected to alternate intervals of immersion in the saline bath and to stimulation while in the moist chamber. As the irritability gradually ap-

<sup>2</sup> 0.7 per cent NaCl plus 0.025 per cent  $\text{CaCl}_2$  plus 0.030 per cent KCl.

<sup>3</sup> For convenience the formula  $S-P$  is adopted to indicate strength of stimuli. For instance, " $S\ 120\ P_2$ " signifies an induction shock of such intensity as is delivered by the Petzolt coil with the secondary 120 mm. from the primary and the primary connected with a cell having a voltage of 2.

<sup>4</sup> This initial stage of inexcitability can be much shortened (1) by treating the strip with a sodium calcium solution, (2) by adding a small amount of atropine to the saline bath, (3) by saturating the saline bath with carbon dioxide, and (4) by raising the temperature of the strip and then stimulating it.

proaches normal, stimuli of  $S\ 160\ P_{2.25}$  intensity are effective when not less than five seconds apart. Extra contractions, if not too frequent, may also result from similar stimuli administered a fraction of a second before the end of a primary systole. Extra stimuli that pass through the muscle earlier than this are ineffective unless the irritability increases. If it increases, not only may the muscle respond earlier in a given diastole, but eventually, with the strength of the induction shock reduced to  $S\ 300\ P_{2.25}$ , it may continue to yield primary contractions regularly, as was the case in other experiments in which a stronger stimulus was used. When maximum irritability is reached, stimuli of  $S\ 200\ P_{2.25}$  or  $S\ 250\ P_{2.25}$  intensity may be effective during any part of the diastolic phase. Should, however, the interval between the primary contractions be shortened, the time would in proportion be lengthened between the end of systole and the first effective stimulus of the following diastole, or the extent of the contraction would be reduced.

Ventricular strips that developed normal irritability and were then subjected to the influence of the Ringer solution for long periods of time, even though not stimulated often, lost their irritability much more quickly than when kept in a moist chamber and moistened occasionally. This has been attributed by some to a lack of oxygen. It is true that by passing oxygen through the solution irritability is improved and maintained longer. Although lack of oxygen is of course an important factor, it is not the only one to be reckoned with, for even in the oxygenated Ringer the muscle after a time ceases to respond normally. It was further observed that even if much care were taken to wash the heart free from blood, the Ringer bath turned opalescent if it were heated. Ringer solution must therefore have some effect in reducing the irritability, perhaps by dissolving out some of the proteids of the strip. Further investigation, however, must be made along this line before any definite conclusion can be reached as to the kind and quantity of proteid dissolved and to just what extent this loss may account for the loss in contractility.

The experiments described in the foregoing paragraphs show the reaction of the heart to stimuli that allow the excitability to develop normally. If, however, the muscle be plied with stimuli of gradually increasing intensity so as to force it to respond, interesting differences may be observed.

For instance, induction shocks of gradually increasing intensity

become effective more and more early in diastole, and after a time strong shocks ( $S_{100}$  to  $0P_{2.25}$ ) at the end of a primary systole result in an extra contraction. Thus it was possible to prove the muscle excitable during that phase of the cardiac cycle which includes a point in late systole and extends throughout the entire diastolic and post-diastolic phases up to the time just preceding the next primary systole. The next point was to observe if a similar condition existed during the systolic phase. For this a strip was tested throughout the entire systole, a voltage of 17 being substituted for the  $2\frac{1}{2}$  volts in the primary. With the secondary at 100 the ventricle responded not only at the beginning of diastole, but also late in systole, and in addition the extent of the contraction was more decidedly reduced than ever, the muscle going into tonus from which it recovered with difficulty. In order to avoid the rapid decrease in the extent of the primary contractions resulting from the strong induction shocks and also to eliminate as far as possible the inhibitory effect they might have upon the excitability of the muscle, recourse was had to the following method: The muscle was stimulated only occasionally with strong stimuli, and the primary stimulus was reduced to such a strength as to be liminal. The second induction shock was then raised to the highest intensity possible ( $S_{0P_{17-20}}$ ). Thus, by allowing the muscle plenty of time to recover and by using as few strong stimuli as possible, the tonus and inhibitory effects were reduced to a minimum. This made it possible for the muscle to be tested a number of times before the extent of the contractions was materially diminished. Strips that yielded optimum contractions did not respond to stimuli during the systolic phase by an increase in the extent of the contraction then in progress, nor did they record a flat-topped cardiogram, but a slight reduction in the extent of the contraction then in progress or of the next primary contraction was very common.

Intense stimuli ( $S_{0P_{17}}$ ) throw a muscle into tonic contraction. With this increased tonus the systoles superimposed upon the tonus wave decrease in extent until the contractions proper appear as mere notches and finally reach the vanishing-point. The muscle usually fails to recover, such strips having been kept under favorable conditions in a moist chamber as long as eighteen hours for recovery without evidence of relaxation or of regaining irritability. The condition closely resembles heat rigor, save that the muscle is not

so hard to the touch. Some strips thus thrown into electrical rigor and subsequently heated to 80° C. or more shorten slightly; others not at all.

Since such strong stimuli as  $S\ 0\ P_{17}$  result eventually in the death of the muscle, it is evident that even when used occasionally they must be injurious. Indeed, much weaker shocks ( $S\ 150\ P_{17}$ ) result in a gradually diminished contractility and a certain amount of tonus, and so it is reasonable to suppose that they, too, are injurious. Certainly it would seem beyond reason to assume the presence of so violent a physiological stimulus in the animal organism. The artificial stimulus must introduce pathological conditions, and unless this factor is taken into consideration, one may be led into serious error in bringing results obtained by violent stimuli to explain a normal physiological phenomenon such as the refractory period.

*Auriculo-sinus preparations.*— After transecting the right and left veins 1 to 2 centimetres from their points of insertion in the auricles, the latter were cut off close to the ventricle. The auricles plus the veins were suspended by passing the electrode hooks through the tips of the right and left auricles respectively.

The sinus along with the auricles beat rhythmically, the former often beating more rapidly than the latter. Records show the ratio to vary as follows: 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, or a long series of sinus beats to only an occasional auricular beat. When the preparation was stimulated by induction shocks, the intensity necessary to call forth extra systoles seemed to depend upon the rate at which the auricles were beating. In some of the preparations beating at the rate of one contraction every five to eight seconds, it was found that  $S\ 240\ P_{2.25}$  was ineffective, but that the auricles responded to a stimulus ( $S\ 220\ P_{2.25}$ ) occurring in mid-diastole. By increasing the intensity to  $S\ 200\ P_{2.25}$  and stimulating early in diastole, the muscle yielded one or more extra systoles. A further increase in intensity resulted in fibrillar contractions that persisted for some seconds and, at times, for some minutes. If strong induction shocks ( $S\ 200\ P_{2.25}$ ) during systole were passed through a preparation yielding optimum contractions, no constant results were obtained such as would indicate an augmentation of the contraction then in progress. There was often, however, *an apparent increase in the extent of the contraction proper because of the tonus such stimuli called forth*. The result of a great number of experiments seems to support the idea that stimuli during the systolic

phase that are too feeble to produce tonus do not increase the extent of the contraction, whereas stimuli that do produce tonus may increase the extent of the contraction, but do not result in superposed contractions if the primary contraction is optimum.

There is another remarkable feature displayed in the reaction of the preparation toward very intense stimuli ( $S_{100}P_{17}$ ). If a strong induction shock is passed through the strip during systole while the auricles are yielding good rhythmic contractions, a change in the systole in progress is usually not perceptible, but the extent

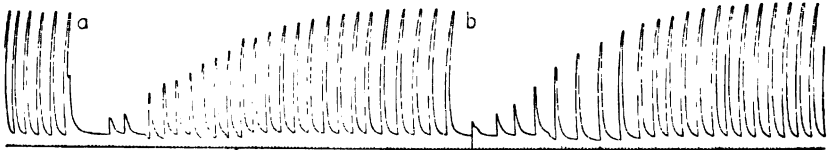


FIGURE 1. About one fourth the original size. Auriculo-venous preparation in Ringer solution. The dots mark the moment of stimulation (single break induction shocks). *a*, effect of stimulating in early diastole with a single strong induction shock ( $S_{100}P_{17}$ ). *b*, effect of stimulus after recovery from *a*.

of the following contraction is very much reduced, accompanied by a slowing of the rate. Starting with the smallest contraction, a series of contractions follow, each successive one of which increases in extent until a maximum is reached (see Fig. 1). If the preparation is stimulated during the diastolic phase, an extra contraction may or may not result; likewise a short so-called compensatory pause may follow the stimuli either in the presence or in the absence of an extra systole. In all cases, however, marked inhibition occurs, followed by a steep treppe.

Treatment by atropine sulphate of the auriculo-sinus preparation brings about results that are noticeably different, as a comparison of *A* and *B* in Table I will show.

The measurements in Table I are taken from a record closely resembling that of Fig. 1. In the left-hand column, 1 *A*, the rate before stimulation was 16.3 per minute; the height of the contraction 57.8 mm. In 2 *A* the muscle was stimulated by a single induction shock, and after a pause of 13.75 seconds the muscle gave a contraction 7.6 mm. in extent with a rate of 13.2 beats per minute. In less than two minutes the contractions increased in extent from 7.6 to 59.3 mm., the rate of the optimum contraction being 13.1 beats per minute. The muscle was again stimulated with the results indicated in 4 *A* and 5 *A*.

The results of *B* were taken from the same preparation after being

TABLE I.

A. In 30 c.c. of Ringer solution.				B. In 30 c.c. Ringer solution + 10 drops of 0.1 per cent atropine sulphate.			
	Time.	Rate per min.	Height of con.		Time.	Rate per min.	Height of con.
			mm.				mm.
1. Before stimula'n	3.48	16.3	57.8	1. Before stimula'n	4.3	22.5	53.4
2. After stimulation	3.48+	13.2	7.6	2. After stimulation	4.3+	19.5	16.0
3. Before 2d stim'n	3.50	13.1	59.3	3. " "	4.4	13.1	57.4
4. After 2d stimu'n	3.50+	9.6	6.4	4. Before 2d stim'n	4.10	11.5	41.2
5.	3.52	13.3	64.4	5. After 2d stimu'n	4.11	18.9	50.6
C. Same as above, at a later stage of atropinization.							
Just previous to stimulation.		Following stimulation.		4.1 atropine added.			
Height of con. in mm.	Interval between con.	Ht. of first spon. con. following pause.	Interval between the higher spon. con. and the con. just preceding it.				
42.2	3.1	48.2	4.8 stim'd in late D., 1 extra con.				
42.2	3.2	45.4	4.7 stim'd in early D., no extra con.				
41.6	3.2	51.2	6.0 stim. in very early D., 2 ext. cons.				
42.2	3.0	49.8	4.6 stim'd in late D., 1 extra con.				
41.8	3.2	45.8	5.1 stim'd in late S., no extra con.				
....	....	....	.... ....				
22.5	2.8	23.7	5.2 stim'd in late S., no extra con.				
22.8	3.2	26.6	4.2 stim'd in early D., 1 extra con.				

treated with atropine in addition to Ringer solution. The muscle was immersed, and in two minutes the rate had increased from 13.1 to 22.5 beats per minute, the height of the contractions being 53.4 mm. Table I 2 B shows the effect of a single induction shock during the diastolic phase, which produced an extra systole followed by a pause of 5.5 seconds. After this pause the first contraction occurred, measuring

16 mm., the rate assumed being 19.5 beats per minute. In less than a minute the extent of the contractions was 57.4 mm., and the rate was 13.1 beats per minute.

The most interesting effect of the solution is that induction shocks did not result in all of the inhibition phenomena shown by the muscle suspended in normal Ringer. The small contraction following the pause in the non-atropinized auriculo-sinus preparation was displaced in the atropinized auricles by a contraction greater in extent than either the spontaneous contraction preceding or following it. As in the normal heart, then, the contraction following the so-called compensatory pause is one of increased extent. In the more advanced stages of atropinization when the force of the contraction has been reduced to less than half its original extent, very intense stimuli during the systolic phase may prolong the pause between the contraction then in progress and the one following.

According to some, atropine paralyzes the vagus endings that are still left in the excised strip and stimulates the accelerator endings, but it is evident that this is not all that happens. The drug acts upon the muscle itself, causing a gradual loss of contractility, especially when the heart tissue is exposed for some time to the direct influence of the atropine-containing solution. It is likewise evident that atropine acts at first as a powerful heart muscle stimulant, this stimulating effect being brought out best in strips of slightly depressed contractility. Often it is possible to secure an increase in rate by as much as 200 per cent of the rate just previous to atropinization, and along with this increased rate and augmentation of the contraction by as much as 800 per cent. The first and primary action of atropine sulphate, then, is that of a muscle stimulant; its secondary effect, when the muscle is under its prolonged influence or when it is given in strong doses, is that of a depressant, but in spite of these marked changes in irritability there seems to be no change in the absolute refractory period.

If the lobes of auricles rather than the auriculo-sinus preparation are exposed to a normal Ringer solution and studied by the method described for the ventricular strip and auriculo-sinus preparation, it will be found that they resemble, on the one hand, the reaction of the ventricular strip in being less rhythmic, and, on the other hand, the sinus in that they are more quick in reaction and more sensitive to strong stimuli. The amount of tonus is less than that shown by the sinus. Thus they may be said to stand, physiologically, midway between the ventricular and the interauricular portion.



If the large veins be used or if their base, including portions of the septum, be properly cut and suspended, vigorous beats can easily be had. Strips from these regions show a refractory period that covers a portion of systole corresponding to that already described for the auricles, interauricular portion, and ventricles (see Fig. 2). These strips are very sensitive to strong stimuli, their

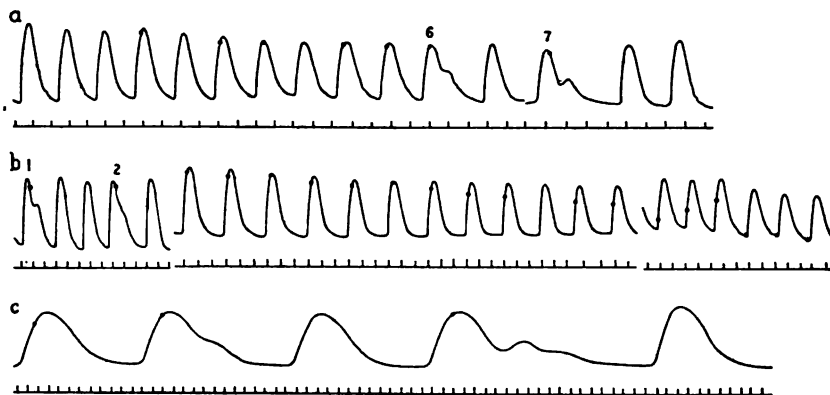


FIGURE 2. About two-thirds the original size. Strips of right vein of *Chelydra serpentina*. The dots record the moment of stimulation (single break induction shocks). *a*, Induction shocks (S 220 P<sub>17</sub>) 6 and 7 effective. *b*, Induction shocks (S 220 P<sub>17</sub>), 1 and 2 effective. Stimuli subsequent to 2 produce inhibition. If the induction shocks have not injured the muscle and the muscle is not again stimulated, subsequent cardiograms soon resemble those first figured. *c*, Stimulus 1 marks the late limit of the refractory period. Stimulus 2, being later, becomes effective.

reactions being primarily inhibitory and tonus phenomena. These phenomena are so prominent that it is often difficult to tell just how refractory the muscle is during systole. This much, however, can be said: (1) that stimuli not strong enough to call forth tonus are wholly ineffective during systole in augmenting the contraction then in progress or in calling forth extra systoles; (2) very intense stimuli (except in late systole) do not result in augmentation or in extra systoles, but almost invariably result in marked tonus and in inhibition of spontaneous beats, these phenomena lasting for some seconds or minutes, depending upon the intensity of the stimuli. As in the auriculo-sinus preparation, there was noticed in rapidly beating strips a decided decrease in excitability towards induction shocks during diastole and very strong stimuli had to be used to elicit extra systoles. I was unable to detect any material

difference in the relative duration of the absolute refractory period of the veins and the sinus region and the other parts of the heart.

Briefly summarizing the results of the foregoing section:

1. When yielding optimum contractions, ventricular and sinus strips, and auricular lobes do not yield augmented or compound contractions in response to induction shocks sent into the muscle before late systole. If stimulated in late systole, compound contractions may result.

2. A single induction shock passing through the preparation during systole may result in:

- (a) A reduction in the extent of a ventricular, auricular, or sinus contraction then in progress, provided the stimulus occur near the middle of the systolic phase or in the early part of it.

- (b) A slowing of the rate of the spontaneously beating preparations of the auricular lobes, auriculo-sinus, and of the right vein (which is often completely inhibited for some seconds), the amount of slowing increasing in the order of the parts named.

3. Stimuli administered at the end of systole or during any part of diastole of the above-named preparations may result in compound contractions. In rapidly beating auriculo-sinus and venous preparations, however, inhibition phenomena only may result or the preparation may be thrown into tonus.

4. Rhythmic induction shocks which at first are subliminal may eventually become liminal and apparently aid the strip to recover normal irritability.

5. Strong induction shocks are injurious, soon reducing the contractility of the muscle and also reducing its excitability towards stimuli that before the administration of strong shocks had been just liminal or barely effective.

6. Very intense stimuli repeated a sufficient number of times produce more or less tonus. As the tonus increases, the superposed systoles become less and less in extent. The amount of the tonic contraction is greatest in the portions of muscle from the region of the insertion of the great veins and decreases in the following order: interauricular portion, base of the auricles, auricle, and ventricle.

7. When ventricular strips are stimulated by very intense stimuli until no further increase in the extent of the tonic contraction takes place, the muscle goes into electrical rigor from which it does not recover. Even if the preparation is heated to 70° or 80°, no further shortening usually results.

The above results indicate (1) that a small part of systole, the whole of diastole, and, if there be one, the post-diastolic pause comprise a period of varying irritability, and (2) that the beginning of systole, extending to the first responsive point in late systole, comprises a period of non-response, an absolute refractory period (see Fig. 3).

Having found an absolute refractory period and a period of reduced irritability in all three parts of the reptilian heart sub-

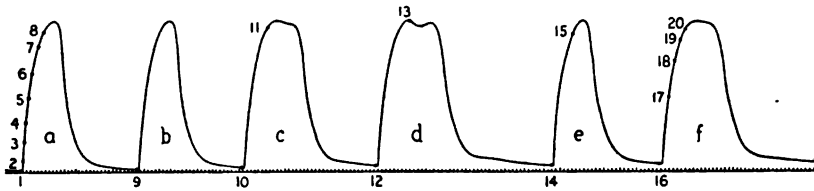


FIGURE 3. Two fifths the original size. Normal ventricle. The dots mark the moment of stimulation (single break induction shocks). Stimuli 2 to 8 inclusive and stimulus 15 ineffective; hence cardiograms *a* and *e* resemble normal cardiogram *b*. Stimuli 11 and 13 effective, bringing about compound contractions *c* and *d*. The absolute refractory period must lie somewhere between point one and point 8 of *a*. The late limit must be slightly earlier than 11 of *c* and later than 8 of *a*. Stimulus 20 must be the effective one in cardiogram *f*.

jected to Ringer solution, I next endeavored to determine to what extent the irritability could be influenced by varying the chemical constituents of the saline bath.

**I. The effect of varying the amount of potassium.**—In order to eliminate as far as possible the cumulative effects of the Ringer solution, the experiments were carried on after this general plan: Strips *a* and *b* from the same ventricle or from corresponding sides of similar ventricles<sup>5</sup> connected in series were placed in separate vessels and suspended in 30 c.c. of Ringer solution, kept at a given temperature by the same water bath, and stimulated by the same break shocks. After determining their liminal stimulus a given amount of potassium chloride was added to *b* at stated in-

<sup>5</sup> Although strips from the same ventricle were for the most part used, they varied in reaction. At 21°–23° C., though there was an increased irritability in both strips, the optimum irritability was developed first in the right ventricular strip. In the left half the cardiogram was flat-topped, suggesting imperfect conductivity, but this peculiarity gradually disappeared, and by the time the strip yielded optimum contractions its cardiograms resembled those of the right half. A tabulation of the height of the contractions shows a gradual increase in the extent of the contractions and a response to extra stimuli increasingly early in diastole.

tervals, after each of which the excitability of both strips was again tested.

*Potassium chloride in less than physiological amounts.*— In general it was found that if the increment of potassium chloride be small its effect is more or less masked by the cumulative action of the Ringer solution, especially when the increment does not exceed 0.002 per cent and when the interval between the doses is prolonged by the time spent in testing the excitability. When 0.005 per cent to 0.010 per cent is added, the effect is shortly seen by a marked influence upon the muscle's response to induction shocks and upon the form and extent of the contraction; that is, there is a decrease in irritability.

*Potassium chloride in excess of physiological amount.*— When the strips were judged to have reached optimal irritability, 0.1 c.c. of potassium chloride solution was added to the 30 c.c. of Ringer solution containing the right half of the ventricle, thus raising the percentage of potassium chloride from 0.03 per cent to 0.046 per cent. After twenty-five minutes the irritability of the strip was tested, but with this exposure there was no perceptible negative change. The change, however, that took place by increasing the amount of potassium chloride to 0.063 per cent was marked. A tabulation of the height of the contractions, mentioned above, does not show all that is revealed in the records taken after the addition of the second tenth of a cubic centimetre of potassium chloride solution, since, besides the gradual decrease in the extent of the contractions as shown in the table, there is a slight reduction in excitability, the diastole is prolonged, and the preparation responds less frequently than before. At this time the right half, treated with potassium chloride, must be stimulated at intervals of longer duration than five seconds in order to respond to each primary stimulus of moderate intensity, whereas the left half without the influence of potassium may respond regularly to stimuli five seconds apart.

It is a peculiarity of strips treated by a percentage of potassium chloride in excess of physiological amount to respond at their best after long periods of rest. They may respond at the beginning of a series of contractions to an extra stimulus 0.7 second after the completion of systole, but in subsequent contractions a stimulus of the same intensity may not be effective unless the interval between the completion of systole and the second stimulation amounts

to three or more seconds. This indicates a sudden drop in the irritability of the muscle after the first contractions. The loss in contractility can be counteracted by increasing the intensity of the stimulus, but if the muscle is forced to respond under these conditions, the extent of the contractions gradually, and often suddenly, diminishes, depending upon the strength of stimuli and the number of effective ones sent into the muscle per unit of time. When the stimulus exceeds  $S_{100}P_{2.25}$ , the preparation is thrown into more or less tonus and the extent of the contractions decreases in proportion to the amount of tonus present. If the amount of potassium chloride is increased to 0.0789 per cent or to 0.0952 per cent, the muscle soon ceases to respond to the original liminal stimulus, and in response to stronger stimuli yields contractions varying in extent, especially if the muscle be stimulated at regular intervals of five to fifteen seconds. The relative extent of the contractions under these conditions may be represented by the following numbers, which show the actual measurement in millimetres of a series of successive cardiograms: 3, 6, 7, 6, 15.8, 20.2, 12.2, 12.1, 20.5, 7.6, 17.2, 9.7, 21.7, etc.

The muscle may also show a decided increase in the length of the latent period, accompanied by partial contractions provided the stimuli are just liminal; that is, a small contraction is recorded which, if followed by a larger one, may or may not blend with the larger contraction. If stronger stimuli are used, the latent period is reduced and the partial contraction disappears, the stimulus calling forth either a maximal contraction or none at all.

Finally, the form of the cardiogram recorded by the potassium strip differs from that yielded by its companion strip in Ringer solution in that it shows a very slow diastole and a slightly prolonged systole, the duration of the systole usually being longer in proportion as the latent period is increased.

The action upon the muscle of potassium above physiological amounts is analogous to that which obtains in a rhythmically beating muscle immersed in sodium potassium solution at  $18^{\circ}$ – $20^{\circ}$  C. or in normal Ringer plus 0.04 per cent or more of potassium chloride; that is, it soon contracts and relaxes more slowly. In addition to this, however, there seems to be a certain degree of tonus brought about in response to strong stimuli.

*Reaction of auricles in sodium potassium and stimulated by strong induction shocks.*—After the auricles had been separated

from the ventricle close up to the latter and the veins removed at their point of insertion into the interauricular portion, they with their connecting part were suspended in the sodium potassium solution, kept at  $20^{\circ}$ , and stimulated while in the moist chamber above the solution by very strong induction shocks ( $S\ 180\ P_{20}$ ). The first result was a rapidly descending treppe. An extra induction shock during the early part of systole brought at first no effect except a reduction in the extent of the contraction, but when

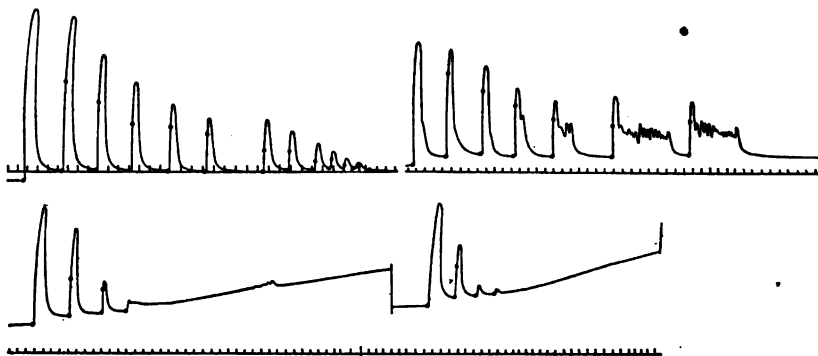


FIGURE 4. Four sevenths the original size. Auriculo-sinus preparation suspended in sodium potassium solution and stimulated ( $S\ 200$  to  $100\ P_{17}$ ) in a moist chamber above the solution. The strong stimuli not only result in inhibition, but also in an after effect, bringing on fibrillar contractions. The dots record the moment of stimulation.

the muscle was stimulated ten or more times and the extent of the contraction had been reduced to less than 50 per cent of its original height, an extra stimulus in mid-systole produced a single extra systole, two or more systoles, or fibrillar contractions (Fig. 4, *a, b, c*).<sup>6</sup>

These results are possible only when very strong induction shocks are used. Moderately strong induction shocks passed through the preparation during systole seem to have no effect other than inhibition, and stimuli that produce no permanent injury to the muscle are effective during diastole only or at best in late systole.

Emphasis has already been laid upon the injurious effect of strong induction shocks. Intense stimuli, as those used in the present case, must produce changes in the muscle that result in intracellular conditions, the effects of which continue to act for

<sup>6</sup> Raising the temperature of the preparation to  $26^{\circ}\text{C}$ . increases the tendency to go into fibrillar contractions and favors tonus. Sodium calcium solution increases the tendency towards tonic and fibrillar contractions.

some time. The moment of sending in the stimulus is recorded, but there can be no direct record of the duration of the intracellular conditions resulting from that stimulus. It is reasonable, however, to suppose that they last long enough to be carried over and excite a contraction of the muscle at a later period. The long latent period that intervenes before the appearance of the extra systole favors such a view. If the direct effect of the induction shock were in evidence, or if there were available material for continuing the contraction, it would seem a natural result at least for the muscle to record a flat-topped curve. On the contrary, the systole in progress is not augmented, but after the lever passes the tip of the curve in its descent, it is either retarded or may record an extra contraction. It seems plausible, then, that the contraction that does take place may be the result of an after discharge within the muscle cells and perhaps within the heart ganglia, brought about, not by a direct stimulus, but by its resulting conditions, which, however, cannot be effective until the physiological processes involving the rearrangement of the colloidal material and the proper adjustment of the ionic charges have taken place in the muscle elements. (For summary of this section see pages 156-157.)

**II. The effect of varying the amount of calcium.**—In the foregoing section it has been shown that primarily potassium diminishes the contractility, augments and prolongs the period of reduced irritability, but does not materially affect the absolute refractory period. Since calcium chloride is known to further the processes favorable to a well-sustained rhythm, the question arose to what extent varying amounts of calcium affect the excitability of the cardiac muscle during the different phases of its contraction cycle.

In order to determine this, strips of ventricle were prepared and suspended (as previously described) in a calcium-free solution kept at 23° C. At the beginning of the experiment the strips showed some difference in their reaction, the strip from the left side responding oftener and yielding contractions measuring 21.8, while those from the right half measured only 16.7 mm. In eighty minutes the extent of the contractions of the right and left halves had diminished to 12.6 and 16.9 respectively, both having in the mean while developed an increased excitability. At first the intensity of the liminal stimulus was only  $S_{320} P_8$ . At the end of eighty minutes, however, the left half responded to  $S_{380} P_8$  five seconds apart, and the right half did not

respond with any degree of certainty even to  $S\ 360 P_5$ . Ascending *treppe* was usually prominent in the right half, at which time the strip responded every five seconds to  $S\ 360 P_5$ , until the contractions had reached a maximum, and thereafter  $S\ 360$  ceased to be effective until after a short period of rest. This initial increase of excitability seems due to the stimulation and not to the potassium chloride solution itself. At the end of three hours the extent of the contractions was 6.8 mm. and 3.8 mm. for the right and left halves respectively. When the excitability had diminished, the secondary was moved up to 300, which caused the right half to respond to stimuli 0.5 second after the systolic phase and the left half 0.4 second thereafter.

Up to this time the strips had been kept under exactly the same conditions, both having been exposed to 30 c.c. of sodium potassium solution. At the end of the third hour, however, 0.0098 per cent of the  $\text{CaCl}_2$  was added to the vessel containing the left half, while the right half was kept in its original solution. The two were stimulated after seventeen minutes with the result that the right half showed a decrease in extent of the contraction from a total height of 6.8 mm. to that of 3.2 mm., and the left half influenced by the added calcium chloride showed an improvement in its contractions from 3.8 to 6.2 mm. So far as I was able to determine, however, there was no increase in the excitability of the strip treated with calcium; if anything, a decrease, since the muscle responded to  $S\ 300 P_5$  one or more tenths of a second later in diastole than before adding the calcium.

At 1.45 P. M., three hours and twenty-five minutes after suspending the strip, the amount of calcium was increased to 0.0197 per cent for the left half. At 2.3 P. M. the strips were re-stimulated. In general, it was noticed that the right half in 0.7 per cent NaCl plus 0.03 per cent KCl showed a slight increase in excitability, but a marked decrease in contractility, whereas the left half in 0.7 NaCl plus 0.0197 per cent  $\text{CaCl}_2$  showed a decrease in excitability and an increase in contractility. More specifically, it was found that the right half showed a decrease in the extent of the contraction from an original height of 3.2 to one of 2.9 mm., and the left half showed a steady increase from 6.2 to 13.2 mm. As before, however, the excitability increased for the right half in response to  $S\ 300$  and decreased for the left half, since the earliest response for the former was 0.32 to 0.39 second after the end of systole and for the latter 0.9 second thereafter. By 2.42 P. M. the extent of the contractions of the right half had decreased from an original height of 2.9 to one of 2.4 mm., while the extent of the contractions of the left half (in calcium solution) had increased from a height of 12.2 to that of 14.3 mm.

In order further to test very small quantities of calcium under the



above-mentioned conditions, enough calcium chloride was added to the solution containing the right half of the ventricle to make the percentage equal to 0.0049 per cent. The strip was exposed to this solution from 2.42 to 3.10 P. M. and at the end of the time the extent of the contractions was increased from a height of 2.4 to 4.0 mm. Longer exposure to the same solution, however, decreased the extent of the contraction to 2.6 mm. Meanwhile the left half had been exposed to a solution containing 0.0295 per cent of calcium chloride and at 3.10 P. M. yielded contractions measuring 27.8 mm. It gradually improved in contractility under the influence of the increments of calcium chloride so that by 3.56 P. M. the muscle yielded contractions measuring 34.6, the concentration of the calcium chloride having reached a percentage of 0.0494.

At 3.57, when the right strip had lost the contractility gained by the influence of the small amount of calcium added at 2.42 P. M., it was immersed in a solution containing 0.0098 per cent, and the left strip was immersed in a solution containing 0.0592 per cent of calcium. At 4.12 the total height of the contractions of the right strip had increased from 2.6 to 5.5, and those of the left from 34.6 to 36.2 mm. Gradual improvement in the contractility of the right ventricle while exposed to the solution containing 0.7 per cent NaCl plus 0.03 per cent KCl plus 0.0098 per cent  $\text{CaCl}_2$  continued until 5.26, at which time its contractions measured 14.3. From this time on its contractions decreased as before, until the addition of fresh calcium chloride again revived the muscle, making it yield contractions surpassing in extent all previous ones. For the left half the amount of calcium chloride was then raised to 0.0690 per cent, and later in response to stimuli yielded contractions 43.4 mm. in extent; the amount of calcium chloride was then raised to 0.1085 per cent, which brought forth no further increase in the extent of the contraction, — on the contrary, a decrease.

From the foregoing it will be seen that percentages of calcium slightly above or below the physiological amount are an important factor in the maintenance of normal irritability. On the one hand quantities of calcium exceeding two to four times the physiological amount may at first bring about a faster rhythm, a decreased amplitude, and a reduced excitability, and may eventually reduce the irritability to zero, whereas, on the other hand, a very low percentage of calcium improves the contractility. This latter effect, however, is not for long. Soon the muscle relapses into its former condition of reduced contractility, and if fresh calcium be not added the contractility gradually approaches zero. The irritability of the strip may be repeatedly revived merely by adding fresh

calcium chloride to the original bath. This would seem to favor the idea that the muscle is excited to contraction not simply by a liberation of free calcium ions within the muscle substance itself nor by their migration out of the muscle, but rather by the introduction of fresh ions into the contractile substance. Thus the results of experiments with small doses of calcium give evidence that in some way the introduced chemical gradually loses its power to favor muscle irritability. In order to explain this it seems plausible to assume that the calcium ions in solution gradually decrease in number by reason of uniting with the proteid molecule or with the products of muscle metabolism. If the number of free ions decreases in the solution, it would be natural to infer that the number within the tissue itself would likewise decrease and the irritability of the muscle would be correspondingly reduced. This hypothesis has been chosen as a working basis, and it is hoped to test its validity by a series of experiments in which the matter is approached from the standpoint of quantitative analysis and physical chemistry.

*Seven-tenths per cent sodium chloride plus 0.025 per cent calcium chloride.* — It is well known that ventricular strips in a sodium calcium solution soon develop an automatic rhythm. Within certain limits the excitability toward induction shocks is also greatly facilitated. It has been pointed out by other writers<sup>7</sup> that calcium shortens the initial "standstill" or "latent period" usually present in freshly cut strips. I find that it has an analogous action in greatly shortening the period during which the muscle responds with partial contractions to induction shocks. At first both contractility and excitability are developed, so that a strip not only contracts with greater force, but weaker and weaker induction shocks are able to call forth these contractions. In like manner the period of reduced irritability during the diastolic phase gradually succumbs to the action of the sodium calcium and the electrical stimuli, causing induction shocks that earlier in the experiment were ineffective before mid-diastole later to effect an extra contraction at the end of systole. Strengthening the stimulus so as to force the muscle to respond to induction shocks in late systole, as already described, in connection with sodium-potassium solutions, diminishes the extent of the primary contractions, increases the tonus if stimulated frequently, and usually throws the strip into inco-ordinated

<sup>7</sup> MARTIN: This journal, 1904, p. 103; 1906, p. 194.

contractions. The nature of the inco-ordinated contractions seems to depend upon the condition of the muscle and upon the strength and time of the stimulus.

There is what may be called a critical period in the diastolic phase, corresponding very nearly to the time of the earliest effective stimulus for a given diastole, at which time a strip in a condition of optimum irritability appears to be demoralized by strong induction shocks ( $S_{100-0}P_2$ ). At such a time a single induction shock often, instead of effecting a single systole, produces two or more extra contractions, and if the stimulus is too strong the muscle yields a longer or shorter series of fibrillar contractions. The tendency to go into fibrillar contractions is increased by stimulating the muscle during successive diastoles. If the stimuli are properly graduated and timed, the strip may at first yield normal extra systoles, but after a number of such responses it often yields double extra systoles in response to single extra induction shocks, and if the stimulation is continued it begins to yield the characteristic vermiform or fibrillar contractions, each series of which comprises individual contractions that invariably measure less than normal optimum contractions. The tonus is usually considerable, depending upon the number of fibrillar contractions per unit of time. As the number increases the tonus curve gradually approaches a straight line; as the fibrillations become slower, the individual contractions seem to fuse into increasingly large waves, until the muscle finally relaxes as a whole and contracts normally.

From these results it would seem that calcium chloride in physiological amounts (0.025 per cent) at first improves not only the contractility but also the excitability of the freshly cut strip. It shows a greater tendency to go into fibrillar contractions than when treated with normal Ringer or sodium-potassium solution. Although the period of reduced irritability is readily overcome by strong stimuli, the absolute refractory period seems not to be shortened beyond late systole. After many experiments I have been unable, even with the strongest induction shocks ( $S_{100}P_{17}$ ), to call forth extra systoles when the stimulus occurred earlier than the beginning of diastole or in very late systole (near the tip of the curve). After exposure to the bath for some time the muscle gradually lost contractility, but this could be temporarily improved by the addition of calcium chloride. Along with the improved contractility there was, contrary to what might be expected, a loss in

excitability, stronger induction shocks being necessary in order to elicit extra systoles in early diastole.

*Sodium chloride plus calcium chloride in excess of physiological amount.* — In another series of experiments the effect of more than the physiological amount of calcium chloride was tried on perfectly fresh hearts. Strips of ventricle were subjected at once to a bath containing three and a half times the physiological amount of calcium chloride in 0.7 per cent NaCl solution. It developed rapidly the contractility of the strips, often to the extent of a quick automatic rhythm, and to provoke extra systoles a strong induction shock had to be used. The rapid rate combined with the effect of the strong stimuli soon resulted in a marked diminution of the extent of the contractions. In the strips yielding sub-optimum contractions very strong stimuli ( $S_{120}$  to  $0P_{17}$ ) were effective in late systole, and it was possible to secure phenomena resembling summated contractions of skeletal muscle. A strip that yields superposed contractions may, in response to a series of properly chosen stimuli, produce compound contractions closely resembling incomplete tetanus (Fig. 5 *a, b, c, d*). It will be noticed that the extent of the first contraction of the series and also the contractions illustrating summation measure much less than the optimum contractions.

The combined influence of calcium and strong stimuli, like an excess of potassium chloride, throws the strip into inco-ordinated contractions. These, as is proven by the following experiment, are not necessarily due to the induction shocks. If a ventricular strip is cut so as to leave one end attached to the auriculo-venous portion of the heart, the ventricle will for a time respond regularly to each auricular contraction, but after having been exposed for some time to the solution containing 0.08 per cent of calcium chloride its different parts beat inco-ordinately, resulting in vermiform contractions. It is, of course, known that a high percentage of calcium chloride produces a partial or a complete block. With the partial block the portions of the strip on either side of it may so beat as to superpose the contractions of one part upon those of the other (Fig. 5 *e*).

It would seem, therefore, that there are two possible kinds of contractions for a given strip, which result in systoles measuring less than optimum contractions of the normal strip: (1) that due to partial or complete block; (2) that in which the strip may yield

a sub-optimum contraction by reason of the fibrils as a whole gradually losing contractility or by the fibrils of one end possessing a greater degree of contractility than those of the other.

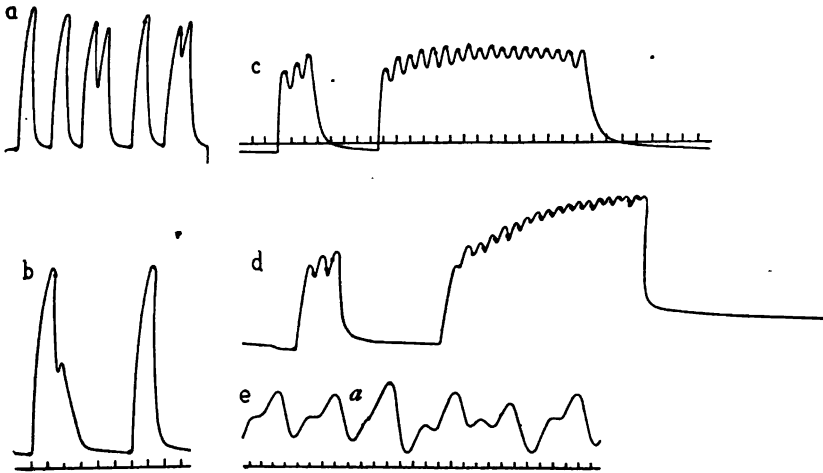


FIGURE 5. About one half the original size. Two ventricular preparations. *a* and *b* nearly normal, taken a short time after immersion in a solution containing an excess of calcium chloride. *c* and *d* represent compound contractions resembling incomplete tetanus, *c* taken from same strip as (*a*) after contractility had been reduced; *d* from same strip as (*b*) after having been treated with an excess of calcium chloride. *e* shows superposed or compound contractions of an imperfectly conducting ventricular strip connected with the rhythmically beating auriculo-sinus preparation treated with excess of calcium chloride in Ringer. Note effect of stimulus *a*. The dots record the moment of stimulation.

It is significant that inco-ordinated contractions occurred more frequently in a ventricular strip that included the whole ventricle without being severed from the frenum than in hemi-ventricular preparations. Not a few times it was possible to detect a distinct difference in the reaction of the two ends of the same preparation, and a test by mechanical stimuli revealed a marked difference in irritability. The contractions of the strip were not only sub-optimum but very irregular, and it was easy to secure superposed contractions like those in Fig. 5*e*. (For summary of this section, see pages 156-157.)

Thus far the discussion has been confined to heart muscle, the temperature of which was kept fairly constant ( $19^{\circ}$  to  $24^{\circ}$  C., the most favorable temperature being  $22^{\circ}$ - $23^{\circ}$ , which was usually the one used). Another series of experiments was carried on in which the temperature was varied.

As is well known, normal heart muscle is very susceptible to a change of temperature. Especially is this true of a muscle that has been kept at  $15^{\circ}$  C. or lower and then heated gradually to  $26^{\circ}$  or to  $30^{\circ}$  C. Owing to the rapid increase in irritability when thus heated, there still remained the possibility of shortening the refractory period by this means if the period is simply one of reduced irritability.

Ventricular strips were exposed to solutions containing (1) 0.7 per cent NaCl plus 0.030 per cent KCl, and (2) to solutions containing 0.7 per cent NaCl plus 0.025 per cent to 0.404 per cent  $\text{CaCl}_2$ . Strips that failed to respond to a given stimulus in late diastole at  $15^{\circ}$ – $19^{\circ}$  were easily excitable by a stimulus of the same strength when the muscle was warmed up to  $23^{\circ}$ – $24^{\circ}$ , and with a slight increase in intensity of the stimulus, often responded early in diastole. I was not, however, able to detect any variation in the absolute refractory period of strips yielding optimum contractions.

Yet interesting and anomalous phenomena are brought out by a series of experiments in which a bath of sodium-calcium was used. Strips from *Chelydra* ventricle were suspended in 0.7 NaCl plus 0.089 per cent  $\text{CaCl}_2$ . Maximum irritability was soon reached with the temperature  $20^{\circ}$  C. The temperature was then raised to  $25^{\circ}$  and the percentage of  $\text{CaCl}_2$  raised to 0.386 per cent. The strips soon showed a marked diminution in the extent of the contractions. Although there was considerable variation in the extent of the contractions, two or more strong stimuli ( $S\ 150\ P_{17}$ ) during systole gave no constant results indicative of an augmentation of the systole in progress. The muscle, however, often responded to such strong induction shocks near the end of systole (see Fig. 6, last con.). With the temperature gradually raised to  $28^{\circ}$  and with the application of still stronger stimuli ( $S\ 100\ P_{17}$ ), the ventricle gave evidence that the induction shock recorded still earlier in systole had at least resulted in an after effect, for although the primary contractions often no longer equalled the normal ones in extent, extra systoles were superposed upon them. As the height of the main contractions diminished, the superposed systoles became more and more prominent (Fig. 6, *c*, *d*, *e*).

The muscle was then allowed a period of rest, after which it was immersed in a solution containing 0.7 per cent NaCl plus 0.020 per cent KCl plus 0.404 per cent  $\text{CaCl}_2$ , warmed gradually to a

temperature of  $30^{\circ}$  to  $33^{\circ}$  C., and stimulated in the warm moist chamber (being raised out of the solution at the time of stimulation). Upon administering very strong induction shocks ( $S \circ P_{17}$ ), the lever, instead of ascending evenly throughout systole, made its

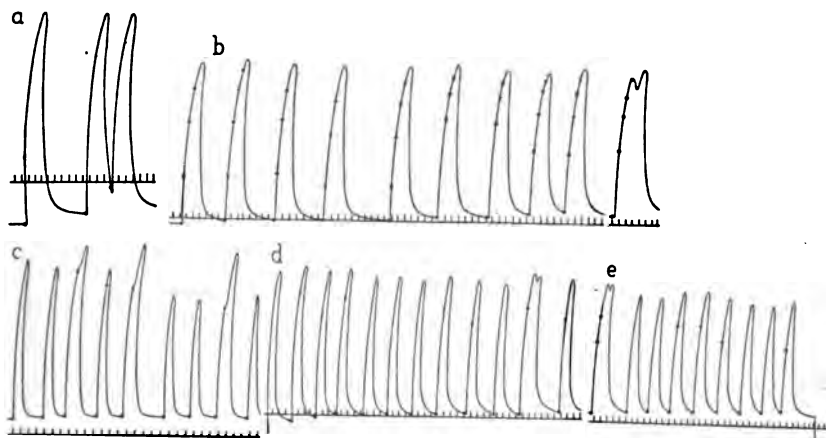


FIGURE 6. One half the original size. The dots mark the moment of stimulation (single break induction shocks). *a* represents ventricular strip with almost normal contraction. *b* shows effect after immersion in Ringer solution containing an excess of calcium chloride (0.22 per cent),  $T\ 25^{\circ}$  C. Strong induction shocks ( $S\ 150\ P_{17}$ ) ineffective except in late systole. *c* is similar to *b*,  $T\ 28^{\circ}$  S  $0\ P_{17}$ . Note superposed contractions due to stimuli in late systole; earlier stimuli result in inhibition. *d* and *e* same as *b*, taken later,  $T\ 30^{\circ}$  and  $33^{\circ}$  respectively, after exposure to Ringer containing 0.24 per cent calcium chloride. The figure does not record the jerky progress of the lever.

transit by a series of jerks, as if the writing-point were being retarded by rough places on the paper. The cause, however, could not be attributed to the recording apparatus, but rather to the muscle itself. It is also significant that the height of the contraction was considerably less than that of the original normal contraction. Along with such a phenomenon it was possible to secure augmented contractions in the latter third of systole by using very strong stimuli; that is, if the muscle received one or more extra stimuli in systole, the contraction when completed might or might not be greater than the one just preceding it. This irregularity may also occur without the extra stimuli, though the latter is more apt to increase the percentage of chance of augmentation. Sometimes the augmentation is followed by an extra systole superposed upon the diastolic limb of the myogram. With ventricular strips of *Pseudemys* warmed gradually to  $30^{\circ}$  C. and stimulated, similar

results may be obtained. When the automatic beats have decreased in extent to less than half their original and optimum height, a strong induction shock in systole may cause the contraction in progress to exceed in extent the one just before it. Likewise a similar stimulus just before the beginning of an automatic contraction may result in a contraction of greater extent than the preceding systole provided the muscle has not been stimulated for some time previously.

Now it is well known that in muscles exposed to solutions or to temperature above optimum the fibres first to lose conductivity are those on the outside. If the temperature be too high, the outermost fibres likewise are the first to lose their irritability and those more centrally located are the last to do so. What is true for temperature is also true for electrolytes, alcohol, etc., not favorable to muscle metabolism. In accordance with this idea, the more sudden the change of temperature, the more apt there are to be inco-ordinated contractions.

In the light of these facts it seems best to attribute the augmentation already described either to a temporary increase in conductivity due to electrical stimuli or to superposed partial contractions, since it is impossible to secure such results in strips the conductivity of which is known to be normal and the fibres of which are approximately of uniform irritability.

Summarizing the results described in the sections devoted to the effects of potassium, calcium, and change of temperature, it may be said that:

1. The effect of KCl when added to 0.7 per cent NaCl plus 0.025 per cent  $\text{CaCl}_2$  or to normal Ringer are masked by the cumulative action of the Ringer or sodium calcium solution; 0.010 per cent or more of KCl, however, has a depressing effect.

2. Calcium chloride in small amounts, 0.0049 per cent, may increase the contractility of muscle, but the increased contractility is soon lost unless fresh calcium chloride is added. The muscle, however, can be repeatedly revived upon the addition of fresh calcium chloride. The excitability toward induction shocks seems not to be increased by the addition of small amounts, especially if the experiment is started with a solution of sodium potassium.

3. Calcium chloride in physiological amounts or somewhat more than 0.025 per cent may at  $19^\circ$ – $22^\circ$  C. rapidly develop the excitability as well as the contractility of a freshly cut strip.



4. Both calcium chloride and potassium in high percentages rapidly decrease the power of contractility. At the same time, if induction shocks strong enough to excite the muscle to contract are used, it is thrown into tonus or may even go into tonus without being stimulated. If the muscle is already passing into tonus by reason of an excess of calcium, the addition of potassium increases the amount of tonus.

5. Excessive amounts of potassium or of calcium or too high a temperature may result in inco-ordinated contractions, especially when strong stimuli are also used.

6. A strip may yield partial contractions on account of the irritability of the contracting fibres being, as a whole, impaired (and then may contract co-ordinately), but under the influence of moderately strong stimuli the conductivity of such a muscle may be temporarily improved, the artificial stimulus often calling forth greater contractions than were the previous spontaneous (sub-optimal) contractions.

7. The fibres of one portion of a strip may show an undeveloped or impaired irritability as compared with other portions, in which case not only do partial contractions result, but they may be more or less inco-ordinated. Under such conditions it is usually possible to secure compound contractions resembling summation and incomplete tetanus of skeletal muscle.

8. A gradual rise of temperature from sub-optimum to  $22^{\circ}$ – $24^{\circ}$  (optimum) increases the excitability and the rate of contractility of the muscle, but does not affect the absolute refractory period.

It is a significant fact that under the influence of strong stimuli, KCl,  $\text{CaCl}_2$ , and variation in temperature, no change takes place in the relation of the absolute refractory period of the normal heart to the duration of systole. It would seem, then, that the absolute refractory period is not influenced by raising the irritability of the muscle. It might at first seem that by increasing the rate of intracellular metabolic changes the muscle ought to respond more and more early in systole. This is, however, not the case, since with the acceleration of the catabolic processes the rate of contraction is hastened and the duration of systole is correspondingly shortened, thus making the absolute refractory period shorter than before, but shorter only by so much as the rate of systole has been increased. In other words, if  $S$  equals the duration of systole

and  $R$  the duration of the absolute refractory period, the ratio  $\frac{R}{S}$  is practically constant for all conditions described in this paper.

With regard to the early literature on the refractory period, the reader is referred to a critical review in an article by Woodworth.<sup>8</sup> As for Woodworth's own conclusions, he finds the refractory period to be absolute. The investigation of which the present article is the outcome was carried on by a modification of his method, and the results obtained, as will be seen from the foregoing summary, coincide, in the main, with those obtained by him. Carlson<sup>9</sup> in a recent article, however, has introduced a new factor, that of inhibition, in considering the refractory period; hence it may be well to consider in brief some of his conclusions.

1. He concludes that the refractory period is merely a condition of greatly reduced irritability, and supports this idea by experiments which show that the muscle responds to stimuli during systole either by augmented or inhibited contractions. Marked inhibition may result if sufficiently strong stimuli be employed. But it has been shown that normal preparations of *Pseudemys*, *Cistudo*, and *Chelydra* heart muscle, when yielding optimal contractions, do not respond to electrical stimuli during any but the latter part of systole. To assume that the refractory period is a condition of reduced irritability because of an inhibitory reaction to stimuli applied during this period is certainly not in accord with the generally accepted idea, described first by Marey,<sup>10</sup> in which the term is meant to imply an absence of extra response in the form of augmented or extra contractions resulting from extra stimuli during this period. Since this idea seems to be the one generally accepted, the fact that the heart muscle during systole may respond to a stimulus in the direction of inhibition should not be allowed to obscure the fact that the heart muscle at this time is refractory to the processes of contraction.

2. In Carlson's experiments upon the dying heart in which he would prove the dependence of the refractory period upon the nervous mechanism alone, he does not seem to take into consid-

<sup>8</sup> WOODWORTH: This journal, 1902, viii, p. 213.

<sup>9</sup> CARLSON: This journal, 1907, xviii, p. 71.

<sup>10</sup> MAREY finally, by reason of an oversight, came to the conclusion that strong enough stimuli did away with the refractory period, but HILDEBRAND later suggested that strong stimuli escaped from the ventricle to the auricle, the contraction wave being then transmitted to the ventricle, which in turn contracted.

eration the change undergone in the conductivity of the heart muscle itself. I have already emphasized the fact that strips long exposed to solutions or otherwise placed under unfavorable nutritive conditions not only lose excitability and contractility, but that certain parts may do so more quickly than others. It would seem that his experiment merely shows that a greater area of contractile fibres has been called into action by the extra stimuli than would otherwise have been reached by the normal excitation wave, or that the strong stimuli temporarily improves the conductivity. It is more than likely that the results represented by his Figs. *A*, *B*, and *C* (*loc. cit.*, p. 55) come under this same head. I am not acquainted with all the conditions of the experiment, but I know that the frog's ventricle is very susceptible to changed conditions of tension, nutrition, etc., and, like the terrapin's heart, readily yields such contractions as might lead one to think the "all or none" law done away with. It seems more than likely that these records were taken from hearts no longer yielding normal and optimal contractions, hence apt to show imperfect conductivity. Finally, tracings taken from the sinus of *Cistudo* (Fig. 7, *B*, p. 83, *loc. cit.* and Fig. 8, p. 85) showing augmentation due to strong induction shocks at the beginning of systole, are not convincing to one who has watched carefully the reaction of great numbers of such preparations. In working with strips one cannot but be struck with the remarkable amount of tonus the venous end of the heart shows. Often the rate of the tonus contraction seems to keep pace with the ordinary contraction, and the resulting tonus curve is so fused into the curve of contraction that all trace of the latter is lost. Such a curve of course can be readily distinguished from a normal curve if the tonus is considerable. But there may also exist a certain amount of tonus that does not reveal itself in an incomplete relaxation before the succeeding contraction, and unless care be exercised, this may easily be mistaken for augmentation of the systole in progress. This condition can often be analyzed by stimulating not simply once but twice or more during the same systole, or by stimulating during alternating systoles, after which it can be noted if the second, third, and later stimuli do not leave some unmistakable trace of tonus. As is well known, excessive tonus is more readily called forth by repeated than by single induction shocks, and the stronger the shocks, the more apt there is to be tonus. I am inclined to think that Carlson has not laid sufficient

emphasis upon this factor, and therefore is somewhat in error in interpreting his records.

Of the hundreds of records made by the author there is none taken from a normally conducting muscle that shows an effective stimulus earlier in systole than just back of the tip of the cardiogram (see Fig. 3). By effective stimulus is meant one that produces either augmentation of the contraction in progress or an extra systole. It seems in accord with experimental data, therefore, to consider this a period during which the muscle cannot respond to stimuli, and it is believed that there is a refractory period of the muscle itself which is due (1) to the lack of completeness of the chemical reaction before another contraction takes place, and (2) to the time necessary for the physical rearrangement of the colloidal particles involved. This does not exclude the idea that the intrinsic heart ganglia are in a similar condition; indeed, analogy would lead us to assume a condition for them similar to that existing in other parts of the nervous system where a refractory period is known to exist.<sup>11</sup>

Howell assumes<sup>12</sup> that there exists within the muscle a store of energy-yielding material that is not dissociable by external stimuli, but which by a series of reactions is transformed into a dissociable compound in definite quantities and at a given rate for a given condition; that upon the initiation of each systole the amount of this dissociable material becomes *nil*, and so long as it is *nil* just so long does the muscle remain refractory to stimuli.

Experimental data support the idea that sodium, potassium, and calcium salts are highly important, if not essential, inorganic constituents in maintaining conditions favorable to contraction. I am of the opinion that sodium and calcium salts by some means aid in the reaction necessary to the formation of this dissociable material. The experimental data at hand suggest the idea that the

<sup>11</sup> According to SHERRINGTON ("Integrative action of the nervous system," 1906) the refractory period for the nerve fibres is less than 0.001 of a second; for the extensor thrust reflex arc as long as one second; for the swallowing reflex arc of the narcotized cat (ZWAARDEMAKER) about half a second. Just how long the refractory period lasts in the intrinsic nerves of the vertebrate heart is not known, but certainly the variation in the different parts of the nervous system is sufficiently great to cover at least the shortest duration of the refractory period found, for instance, in the turtle's heart.

<sup>12</sup> HOWELL: Journal of the American Medical Association, 1906, xlii, Nos. 22-23.

calcium ions in physiological amounts may act as accelerators in the production of the dissociable material, or at least act as an intermediary body active in the changes antecedent to contraction. If the calcium be in too great excess, it probably enters into fixed combination with certain of the intracellular constituents involved in the metabolic processes, and in so doing contraction is either hindered or rendered impossible.

Furthermore, it is conceivable that there are, during the different stages of the normal chemical transformation of the non-dissociable to the dissociable material, conditions that favor a varying degree of ease with which the muscle may be made to contract. In other words, in the early stages of the chemical reaction more energy may be required to decompose the imperfectly transformed material to produce an extra contraction. Such a condition is comparable to the one known to exist during diastole, in which the muscle shows a period of variable irritability, being difficult to excite in early diastole and progressively more easy to excite as it is stimulated nearer the time for the next spontaneous contraction.

It is evident, then, from the experimental data at hand that such a theory as that of Howell's chemical theory of the heart beat covers most satisfactorily the fundamental points with reference to the refractory period. As a working basis for experiments now in progress, this theory, with certain modifications, will be used; that is, it is assumed that the absolute refractory period is due primarily to an intracellular chemicophysical condition that does not admit of further contraction during systole, because (1) all of the dissociable material is used up, with none other available until late systole, and (2) there is a rearrangement of the colloidal particles commensurate with the degree of relaxation and readiness for another contraction.

#### CONCLUSIONS.

1. The absolute refractory period continues to bear a constant relation to the duration of systole, whether the agents used increase or decrease the irritability of the muscle. In other words, if  $S$  equals the duration of systole and  $R$  the absolute refractory period, then the ratio  $\frac{R}{S}$  is approximately constant.
2. It is suggested as a possible explanation of the absolute re-

fractory period that upon the initiation of a contraction all of the dissociable material is used up, and that the colloidal particles undergo a change in size and position, and that so long as these conditions obtain it is impossible for the tissue to contract.

3. Experimental data suggest the idea that the inorganic salts of Na, Ca, and K play an important part in the reforming of the dissociable material, and it is probable that Ca in physiological amount in some way acts as an accelerator.

# THE INFLUENCE OF COLD AND MECHANICAL EXERCISE ON THE SUGAR EXCRETION IN PHLORHIZIN GLYCOSURIA.

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AT the Congress for Internal Medicine, held in 1905, Lüthje<sup>1</sup> made the somewhat astonishing statement that the production of sugar in dogs diabetic after pancreas extirpation varied with the external temperature to which the animal was subjected. Thus, in a dog placed in a cold room the sugar excretion was 47 gm., whereas the same dog in a warm environment yielded only 4 gm. of urinary sugar. Lüthje explained how cold influenced the organism to produce more sugar, the combustion of which could then easily maintain the body temperature. Warmth, on the contrary, reduced the necessity for sugar production, for less heat was needed by the organism. These experiments, however, did not remain long without destructive criticism. Thus Brasch<sup>2</sup> found that exposure to cold had no influence upon the sugar excretion in phlorhizin diabetes in dogs. He explained Lüthje's results by the fact that the dogs had only partial diabetes, due to partial extirpation of the pancreas, and consequently cold could throw sugar formed from glycogen into the blood stream.

A discussion of this subject again took place at the Congress of Internal Medicine in 1907, where Lüthje<sup>3</sup> presented certain blood analyses in support of his claims. Minkowski,<sup>4</sup> however, attacked the position taken by Lüthje, and cited the experiments (still unpublished) performed by Allard in his laboratory upon depancreatized dogs, operated upon by Minkowski himself, in which the urinary

<sup>1</sup> LÜTHJE: *Verhandlungen des Congresses für innere Medizin*, 1905, p. 268.

<sup>2</sup> BRASCH: *Münchener medizinische Wochenschrift*, 1906, vii, No. 17, p. 805.

<sup>3</sup> EMBDEN, LÜTHJE, and LIEFMAN: *HOFMEISTER'S Beiträge*. 1907, x, p. 265.

<sup>4</sup> MINKOWSKI: *Verhandlungen des Congresses für innere Medizin*, 1907, p. 272.

D : N ratio was 2.8 : 1, that is, in which total pancreatic diabetes was present. Under these circumstances alteration in the temperature of environment was without effect. Minkowski's explanation of Lüthje's findings is the same as that of Brasch, that sugar retention and glycogen storage in partially diabetic dogs explain the effects of cold which were obtained. Mohr<sup>5</sup> reports similar findings.

It seemed that these experiments might well be repeated using phlorhizin diabetes as a basis. Brasch did not use a procedure calculated to obtain a total phlorhizin diabetes with a D : N ratio of 3.65 : 1.

The immediate object of this research was to subject completely phlorhizinized fasting dogs to the influence of cold and of mechanical work, thereby greatly raising their fat metabolisms. If under such circumstances the D : N ratio remains unaltered, one can be certain that increased fat katabolism does not involve increased sugar production.

#### METHOD.

It appears to the writer that many researches in which phlorhizin glycosuria forms the method are being accomplished without the clear-cut results which should be attached to them. To obtain the D : N ratio one proceeds as follows: Merck's phlorhizin is used. This preparation is usually a good one, but sometimes its administration causes convulsions and death. Two grams are dissolved in 25 c.c. of a 1.2 per cent sodium carbonate solution by warming to 40°-50° over a water bath, and this liquid is injected subcutaneously through a sterile needle. The dogs may remain without any rise in body temperature throughout a long experiment if so treated. These injections should be made three times daily. In general one may say that the injections must be made every eight hours. The first day's urine is always rich in body sugar, and the D : N ratio is high. The second day the ratio may or may not be established, and this is a day of rising protein metabolism. These two days' urine are therefore discarded.

To relieve the operator, the following procedure, as partly outlined by Stiles and Lusk,<sup>6</sup> may be adopted :

<sup>5</sup> MOHR : *Zeitschrift für experimentelle Pathologie*, 1907, iv, p. 910.

<sup>6</sup> STILES and LUSK : *This journal*, 1903, x, p. 78.



First day 2 gm. phlorizin at 8 A. M. and 6 P. M.; second day, phlorizin at 8 A. M., 3 P. M., and 10 P. M.; third day, phlorizin at 8 A. M.; catheter and bladder washed with warm sterile water through a sterilized catheter at 10 A. M. The urine collected from 10 A. M. onwards will show the D : N ratio as 3.65 : 1. *The interval between 8 A. M., when phlorhizin is renewed, and 10 A. M., when the research day begins, allows the removal of any sugar accumulated in the organism during the long night period.* This procedure permits a comfortable night's rest to the worker. The combined urines of the periods from 10 P. M. to 8 A. M. + 8 A. M. to 10 A. M. will show the usual D : N ratio after the second day of the glycosuria.

One injection daily does not suffice, nor do two injections at the beginning and ending of an ordinary laboratory day. Two-gram doses should be given to a dog. The dogs used should be healthy, their urines free from albumin, and they must not have been treated with drugs which affect the kidney.

All analyses were made in duplicate, sugar by Allihn and nitrogen by Kjeldahl.

#### THE INFLUENCE OF COLD.

An experiment which indicates the discharge of "extra sugar" from the glycogen supply of a fasting phlorhizinized dog through the influence of cold is shown in Table I.

In this experiment on March 4 the dog shivered in the cold of a north wind and produced a total extra sugar elimination of 8.47 gm. of dextrose. The "extra sugar" is obtained by multiplying the nitrogen output of a period by 3.65. All sugar above this quantity is derived from sources other than the proteid metabolism of the period.

It must be recalled that dextrose administered *per os*<sup>7</sup> or subcutaneously<sup>8</sup> is completely eliminated as extra sugar in phlorhizin glycosuria. Therefore any dextrose elimination above the constant ratio — in other words, any "extra sugar" — represents the total of dextrose furnished to the blood in metabolism from sources other than protein. If fat were convertible into dextrose, this should appear as extra sugar. On the second day exposure to cold pro-

<sup>7</sup> REILLY, NOLAN, and LUSK : This journal, 1898, i, p. 400.

<sup>8</sup> STILES and LUSK : This journal, 1903, x, p. 75.

duced an elimination of extra sugar equal to 6 gm. of dextrose. The 8.47 gm. of dextrose have a calorific value of 31.8 calories, or 1.7 calories per kilogram: the 6 gm. = 22.5 calories, or 1.3 per kilogram. Rubner<sup>9</sup> has shown of a small dog weighing about 4 kg. that reduction of the temperature of the environment from 20° to 7.6° may change the heat production from 14 calories per

TABLE I.

Illustrating discharge of extra sugar through cold. Dog I. Setter bitch, had fasted three days. Diabetic 1 day. Weight on March 3 = 18.4 kg. 2 gm. phlorhizin every eight hours.

Date.	Day of diabetes.	Period.	Temp. of room.	Urines.	D.	N.	D : N.	Extra D.	D per hour.	N per hour.
1908		hrs.	degrees.	g.o.						
March 3	2	24	20	575	65.34	17.93	3.64	....	2.72	0.75
March 4	3	6	20	204	18.35	4.97	3.69	....	3.06	0.83
	3	8	8-3 <sup>1</sup>	360	29.60	6.63	4.47	5.40	3.70	0.83
March 5	3	10	16-20	328	29.08	7.10	4.09	3.07	2.91	0.71
	4	8	5-8 <sup>2</sup>	445	26.46	6.11	4.33	4.16	3.31	0.76
	4	6	16-18	255	19.46	4.83	4.03	1.84	3.24	0.80
March 6	4	?	16-18	141	11.20	2.87	3.87	....	....	....
<sup>1</sup> Exposed to cold for first six hours of period. <sup>2</sup> Exposed to cold for seven and one-half hours of period.										

kilogram to 23.1 calories (calculated for a six-hour period). There is in this case an increased combustion of fat amounting to 60 per cent which supplies the necessary heat to the organism as represented by a rise of 9.1 calories per kilogram. The largest extra sugar elimination in an 18 kg. dog would have been insufficient to provide the extra energy requirement of Rubner's 4 kg. animal. Although the exposure to cold on March 4 was longer than on March 5, the extra sugar eliminated was less, which indicates an exhaustion of a glycogen reserve. The total elimination of extra sugar was 14.47 gm. as effected through cold. In this connection

<sup>9</sup> RUBNER: *Die Gesetze des Energieverbrauchs*, 1902, p. 105.

the fact may be recalled that Prausnitz<sup>10</sup> has reported that a dog weighing 22 kg. after fasting twelve days and after excreting 287 gm. of sugar brought about by phlorhizin injections still contained 25 gm. of glycogen in his body.

It is interesting to note that, as in Rubner's normal dog, so also in the diabetic, variations in external temperature are without influence on protein metabolism.

The next experiment illustrates an example of a preliminary elimination of dextrose from glycogen during a first period of exposure to cold, an effect which did not occur during a second period of exposure.

TABLE II.

Illustrating no discharge of extra sugar on second exposure to cold. Dog II. Short-haired bitch. Had fasted one and a half days. Weight on February 14 = 9 kg. 2 gm. of phlorhizin every eight hours.

Date.	Day of diabetes.	Period.	Temp. of room.	D.	N.	D : N.	Extra D.
1908		hrs.	degrees				
Feb. 10-11	..	24	....	....	2.88	....	
Feb. 11-12	1	24	14-21	28.06	5.25	5.53	
Feb. 12-13	2	24	14-21	35.01	9.04	3.76	
Feb. 13	3	6	19	9.47	2.84	3.34	
Feb. 13	..	8	6-9 <sup>1</sup>	16.18	3.63	4.45	4.44
Feb. 14	..	10	16-18	16.21	4.00	4.05	
Feb. 14	4	8	10 <sup>2</sup>	14.06	3.87	3.63	
Feb. 14	..	6	17-19	9.13	2.62	3.49	
<sup>1</sup> Exposed for first six hours to a cold fog. <sup>2</sup> Exposed for six and three-quarters hours to cold fog; melting snow.							

Here, then, the cold fog on one diabetic day led to the elimination of extra sugar amounting to 4.44 gm., while *the same cold on the following day showed the maintenance of the normal D:N ratio without the slightest elimination of extra sugar.*

Another experiment on the same dog at another date showed similar results, as appear below.

<sup>10</sup> PRAUSNITZ : Zeitschrift für Biologie, 1892, xxix, p. 168.

TABLE III.

Dog II. Had fasted four days. Diabetic three days. Exposed to cold (8°-13°) for nine hours on December 26. Weight = 12.8 kg.

Date.	Day of diabetes.	Period.	Temperature of room.	D.	N.	D : N.
1907 Dec. 27	4	hrs. 8½	degrees 8 <sup>1</sup>	15.14	4.41	3.44
Dec. 28	4	10	18-20	17.98	4.89	3.67
<sup>1</sup> Exposed for the first six hours of the period.						

During the night of December 28-29 the bitch whelped three puppies, all dead and about two weeks from term.

In all the cases of these experiments on dogs the cold was sufficient to produce shivering.

It is evident, however, that the chemical regulation of temperature — i. e., the increased heat production necessary for adaptation to external cold — has no effect on sugar production in the fasting organism, except in so far as body glycogen may be converted into dextrose.

#### THE INFLUENCE OF MECHANICAL WORK.

Exact experiments regarding the effect of mechanical work in the diabetic condition have not come to the writer's knowledge. An inaugural dissertation by Kalmus,<sup>11</sup> in which he describes a diminution of the sugar excretion in himself during periods of exercise when under the influence of phlorhizin as contrasted with similar periods without exercise, are not valid for our purpose, for the influence of the drug was frequently absent, the diabetic condition was not total.

Several years ago the writer<sup>12</sup> produced convulsions in phlorhizinized rabbits having a ratio D : N = 2.8 : 1 with the result of an immediate rise in the ratio to as high as 5.7 : 1. He interpreted this result as due to the removal of the glycogen-rest within the animal,

<sup>11</sup> KALMUS: Ueber den Einfluss der Muskeltätigkeit und des Opiums auf die Zuckerausscheidung bei Phlorhizin-Glykosurie, 1906, Dissertation, Halle-Wittenberg.

<sup>12</sup> LUSK: Zeitschrift für Biologie, 1898, xxxvi, p. 111.

for Zuntz<sup>13</sup> had shown that a chloralized rabbit treated with phlorhizin could only be made glycogen-free by strychnin convulsions.

In a later research by Mandel and Lusk<sup>14</sup> on a fasting dog who had a combination of phlorhizin and phosphorus poisoning, the animal had two severe convulsions on one day; his D : N ratio rose to 3.91 : 1, contrasting with 3.61 : 1 on a previous day and 3.71 : 1 and 3.61 : 1 on the two days succeeding. Here, then, the same explanation avails as in the rabbit, — a conversion of glycogen into dextrose and its elimination as extra sugar.

TABLE IV.

Illustrating discharge of extra sugar as result of work. Dog III. February 18, 1907.  
Fasting four days, diabetic two days. Two-hour periods. Weight = 9.0 kg.

Period.	Distance in wheel in metres.	D.	N.	D : N.	Extra D.
Rest . . . . .	....	2.552	0.775	3.43	....
Work . . . . .	750 <sup>1</sup>	4.339	0.907	4.78	1.25
Rest . . . . .	....	2.662	0.794	3.34	....
<sup>1</sup> This work was done during the first half-hour of the period.					

It seemed that more systematic research might throw light upon this subject. Phlorhizinized fasting dogs were therefore made to do mechanical work, and the effect on the urinary D : N observed.<sup>15</sup>

The exercise was accomplished in a "fatigue wheel" designed by my colleague, Dr. Frederic S. Lee,<sup>16</sup> of Columbia University, and kindly loaned to me for the occasion. The wheel had a circumference of 3 metres and was forced by machinery to rotate 20 revolutions per minute. A dog within the wheel therefore travelled during a five-minute interval of time a distance of 300 metres. Since fatigue comes on more rapidly in the diabetic than in the normal muscle,<sup>17</sup> it was found necessary to confine the work to

<sup>13</sup> ZUNTZ : *Archiv für Physiologie*, 1893, p. 378.

<sup>14</sup> MANDEL and LUSK : *This journal*, 1906, xvi, p. 136.

<sup>15</sup> LUSK : Preliminary report, *this journal*, 1906, xviii, p. xii.

<sup>16</sup> LEE : *Proceedings of the Society for Experimental Biology and Medicine*, 1905, iii, p. 15.

<sup>17</sup> LEE and HARROLD : *This journal*, 1900, iv, p. ix.

five-minute intervals and allow five minutes for rest. Otherwise the hind legs dragged in the wheel and the animal seemed in danger of collapse. At the end of a five-minute period of work the dog always seemed fatigued, but recuperated easily during the period of rest. After the above fashion the experiments in Table IV were accomplished.

The resting D : N ratio is slightly under the usual, possibly because only 1 gm. of phlorhizin was administered every eight hours.

Another similar experiment in which a much longer distance was travelled is shown in the following case.

TABLE V.

Dog IV. March 21, 1907. Fasting six days, diabetic two days. Two-hour periods.  
Weight = 6.75 kg.

Period.	Distance in wheel in metres.	D.	N.	D : N.	Extra D.
Rest . . . .	....	2.24	0.661	3.40	....
Work . . . .	1800 <sup>1</sup>	3.70	0.865	4.62	0.707
Rest . . . .	....	2.49	0.709	3.51	....
<sup>1</sup> This work was done during the first hour of the period.					

In both of these cases a certain amount of extra sugar was eliminated as the result of work, but this was very small in amount, and not proportional to the work accomplished. It can easily be explained by a discharge of dextrose from glycogen by the tissues.

That this is true is shown by the following experiment. A dog was prepared by washing him in cold water on the first day of diabetes. On the second day of diabetes he ran 900 metres in the wheel, was given a cold bath, and while he was wet was put in a cold room at a temperature of 10° where he was let shiver for three hours. Then followed this experiment on a third day (see Table VI).

During the first period of work there was a small excretion of extra sugar (0.34 gm.), but *during the second period of work there was absolutely no change in either the dextrose or nitrogen elimination.*

Zuntz<sup>18</sup> finds that a dog weighing 10.57 kg. requires 43.5 calories of energy per kilogram in twenty-four hours and an energy equivalent of 0.66 kilogrammetre to move 1 kg. of body weight 1 metre through space. From this it may be calculated that the diabetic dog required 1.81 calories per kilogram per hour while at rest, and in addition to this the equivalent of 999 kilogrammetres ( $0.66 \times 1500$ ) while at work, or an additional energy equivalent of 2.35 calories per kilogram.

TABLE VI.

Illustrating effect of work in a dog freed from glycogen by cold. Dog V. Fasting six days, diabetic two days. 2 gm. phlorhizin every eight hours. Two-hour periods. Weight = 9.9 kg.

1908. April.	Day of diabetes.	Distance travelled in wheel in metres.	D.	N.	D : N.
1	3	....	4.20	1.19	3.53
....	....	1500 <sup>1</sup>	5.32	1.36	3.90
....	....	....	4.57	1.26	3.63
....	....	1500 <sup>1</sup>	4.62	1.26	3.67
....	....	....	2	2	....
2	4	....	4.14	1.14	3.63
<sup>1</sup> This work was done during the first hour of the period. <sup>2</sup> Interval of ten hours.					

*It is therefore apparent that an amount of work capable of more than doubling the fat metabolism has no effect whatever on the sugar output in a case of total phlorhizin glycosuria. Hence sugar is not derived from fat in metabolism.*

A minor point which is seen in all these experiments, except in the last instance, is a rise in protein metabolism during the period of mechanical work. But in the last instance, where no extra carbohydrate is thrown out into the circulation, there is no rise in nitrogen elimination.

Analogous to this are the findings of Frentzel<sup>19</sup> regarding the influence of work on the metabolism of fasting dogs. The differences between the nitrogen elimination during work and rest were

<sup>18</sup> ZUNTZ : PFLÜGER'S Archiv, 1903, xcv, p. 202.

<sup>19</sup> FRENTZEL : PFLÜGER'S Archiv, 1897, lxxviii, p. 212.

at first very marked, but on a third day of work and an eleventh of fasting a difference scarcely existed.

One can explain this phenomenon by the experiments of Murlin,<sup>20</sup> who demonstrated a considerable retention of glycocoll when this was ingested with carbohydrate,— a retention which, however, was not permanent, but depended upon the presence of the carbohydrate. In like manner the removal of a rest of glycogen by work may bring about an elimination of nitrogen formerly belonging to compounds loosely combined with glycogen.

Another point of interest is that in total phlorhizin glycosuria after the removal of "extra sugar" by work the organism does not retain any of the sugar produced in the subsequent period of rest. This accords with the investigations of Bang, Ljundahl, and Bohm,<sup>21</sup> which show that the power of the ferment which converts glycogen into dextrose is perfectly normal in phlorhizin glycosuria, although the reversible action is entirely in abeyance.

#### GENERAL DISCUSSION.

The writer has labored upon the subject of phlorhizin glycosuria for many years. He believes he has established the general validity of the ratio  $D:N = 3.65:1$  in fasting and meat-fed dogs. He has shown that this ratio does not vary after ingesting fat, and he now adds a further observation that cold and mechanical work which largely increase the combustion of fat may under proper conditions be without influence on the  $D:N$  ratio. All these facts prove that while sugar is derived from protein it is not derived from the metabolism of fat. The ratio also is not dependent upon the size of the dog, for the same ratio obtains in animals varying from 7 to 40 kg. in weight.

This discussion does not exclude the possibility that after *large* fat ingestion a certain quantity of dextrose may be formed from the quickly absorbed glycerine component of fat. But this result has never been seen in this laboratory. Thus Mandel and Lusk<sup>22</sup> gave a phlorhizinized dog 50 gm. of meat and 100 gm. of fat on a day when the dog burned 69.5 gm. of fat, and yet the  $D:N$  ratio remained at 3.61:1.

<sup>20</sup> MURLIN: This journal, 1907, xx. p. 250.

<sup>21</sup> BANG, LJUNDAHL, and BOHM: HOFMEISTER'S Beiträge, 1907, x, p. 312.

<sup>22</sup> MANDEL and LUSK: This journal, 1903, x, p. 55.



There are two points which to-day require vigorous correction. The first correction is that the D:N ratio of 4.4:1, as given by Rubner<sup>23</sup> and now widely quoted, is based upon a single erroneous calculation. Rubner's phlorhizinized dog had a high ratio on the first day of diabetes, as is always the case; the second day the ratio was 2.8:1, and on the third day the dog died. Rubner averaged the ratios of the first and second days and obtained 4.4:1 as a result. The writer does not deny the possibility of a larger production of sugar from the protein of meat than is indicated by the ratio 3.65:1, but he does deny the validity of Rubner's calculation.

Falta and Gigon,<sup>24</sup> after giving casein in a case of human diabetes, calculate the D:N ratio at 4.22:1. This is a different proposition, for casein contains no extractive nitrogen, and contains a different set of amino acids than meat protein. The cases are therefore not comparable.

The second and much more serious correction is concerned with the widely quoted work of Hartogh and Schumm,<sup>25</sup> in which they obtained ratios as high as 9:1 and even 13:1 after fat ingestion in phlorhizinized dogs. The writer does not hesitate to declare these results to be impossible of achievement in well-conducted experiments.

The writer acknowledges with thanks the co-operation of Mr. H. P. Mencken in the accomplishment of this research.

<sup>23</sup> RUBNER: *Gesetze des Energieverbrauchs*, 1902, p. 307.

<sup>24</sup> FALTA and GIGON: *Zentralblatt für die Physiologie und Pathologie des Stoffwechsels*, 1907, ii, p. 242.

<sup>25</sup> HARTOGH and SCHUMM: *Archiv für experimentelle Pathologie und Pharmakologie*, 1900, xlv, p. 2.

## THE PRODUCTION OF SUGAR FROM GLUTAMIC ACID INGESTED IN PHLORHIZIN GLYCOSURIA.

By GRAHAM LUSK.

[From the Physiological Laboratory of the University and Bellevue Hospital  
Medical College.]

THE preceding paper has demonstrated, if further demonstration were needed, that the sugar produced in phlorhizin glycosuria is derived from the metabolism of protein and not from fat.

It has been very definitely shown that this sugar is not derived by a direct cleavage of the protein into a carbohydrate portion and a nitrogen-containing portion, as was at one time maintained. The origin of the sugar is synthetic from various amino acids, as was first predicted by Kossel<sup>1</sup> and Friedrich Müller.<sup>2</sup>

Knopf,<sup>3</sup> at the suggestion of Hans Meyer, made the first experiment in this direction. He gave 50 gm. of asparagin to a phlorhizinized dog. Although the diabetic D:N ratio did not show a total diabetes, it is still possible to calculate that for every gram of the 10.6 gm. nitrogen ingested at least 1.3 gm. of dextrose appeared in the urine.

The experiments next published were by Stiles and Lusk,<sup>4</sup> who gave a digest of amino acids prepared by the pancreatic proteolysis of meat to a completely phlorhizinized dog. For each gram of nitrogen ingested 2.4 gm. of sugar were eliminated in the urine.

Embden and Salomon<sup>5</sup> have given asparagin, glycocoll, and alanin to partly depancreatized dogs, and have noticed large increases of urinary sugar following the ingestion of these substances.

<sup>1</sup> KOSSEL: Deutsche medizinische Wochenschrift, 1898, p. 58.

<sup>2</sup> MÜLLER and SEEMAN: *Ibid.*, 1899, p. 209.

<sup>3</sup> KNOPF: Archiv für experimentelle Pathologie und Pharmakologie, 1903, xlix, p. 135.

<sup>4</sup> STILES and LUSK: This journal, 1903, ix, p. 380.

<sup>5</sup> EMBDEN and SALOMON: HOFMEISTER's Beiträge, 1904, v, p. 507, and 1904, vi, p. 63.

Since the diabetes was only partial, quantitative relations cannot be calculated from these experiments.

Baer and Blum<sup>6</sup> investigated the influence of many substances, including glycocoll, alanin, and glutamic acid, on the acidosis in phlorhizin glycosuria. Since, however, no attempt was made to produce a total diabetes, the results are not valuable for the present discussion.

Glaessner and Pick<sup>7</sup> also report experiments in which they gave various amino acids to phlorhizinized rabbits. They find no con-

TABLE I.

Influence of glutamic acid. Dog V (continuation of experiment on Dog V, this journal, this volume, p. 171). Fasting seven days, diabetic three days. Free from glycogen. Weight, 9.6 kg.

Date.	Day of diabetes.	Period.	Glutamic acid.	D.	N.	D:N	Extra D from glutamic acid.	Glutamic acid. D:N.
1908 April 2	4	hrs. 2	gm. ....	4.14	1.14	3.63	.....	....
	..	6	5 <sup>1</sup> (= 0.47 N)	15.60	3.82	4.10	3.38	6.2
	..	4	....	11.60	3.10	3.74	....	....
	..	11½	....	....	....	....	....	....
April 3	5	2	....	....	0.98	....	....	....
		10	10 <sup>2</sup> (= 0.95 N)	22.82	5.96	3.83	4.53	4.7
<sup>1</sup> Subcutaneously; dissolved in 150 c.c. of a 1.2 per cent Na <sub>2</sub> CO <sub>3</sub> solution. <sup>2</sup> Per os + 38 gm. of lard.								

version of glutamic acid into dextrose in the fasting animal. Unfortunately they injected phlorhizin only once daily and did not obtain a total phlorhizin glycosuria.

It seemed to the writer that the behavior in the diabetic organism of d-glutamic acid, a dibasic monoamino acid with five carbon atoms, would be a subject of interest. Osborne has shown that the protein of meat yields 10 per cent of glutamic acid, while serum albumin yields 7.7, serum globulin 8.5, casein 10.7, and gliadin, the principal protein of wheat, yields 37.3 per cent.

<sup>6</sup> BAER and BLUM: HOFMEISTER'S Beiträge, 1907, x, p. 80.

<sup>7</sup> GLAESSNER and PICK: HOFMEISTER'S Beiträge, 1907, x, p. 473.

Fifty grams of the substance were given to me by a friend who does not wish his name mentioned. The material contained 9.536 per cent N as against 9.523 per cent calculated for glutamic acid. The substance was given *per os* with a little fat or was administered subcutaneously after solution in sodium carbonate.

The preceding experiments were performed (see Table I).

In the above experiment subcutaneous injection of 5 gm. of glutamic acid (= 0.47 gm. N) dissolved in considerable fluid caused

TABLE II.

Influence of glutamic acid. Dog I (same Dog I as mentioned in this journal, this volume, p. 166). April 7 = third fasting day. April 8 = first diabetic day.

Date.	Day of diabetes.	Weight.	Period.	Glutamic acid.	D.	N.	D : N.	Extra D from glutamic acid.	Glutamic acid D : N
1908 April 7		kg. 15.7	hrs. 24	gm. ....	....	3.80			
April 10	3	....	2	....	3.79	1.02	3.70		
			6	10 <sup>1</sup> (= 0.95 N)	19.44	4.17	4.66	} 8.75	9.2
			4	....	10.01	2.45	4.09		
			12	....	24.84	6.99	3.55		
April 11	4	15.1	12	20 <sup>2</sup> (= 1.9 N)	32.22	7.35	4.22	} 13.46	7.1
			12	....	24.71	6.46	3.82		

<sup>1</sup> Subcutaneously; dissolved in 110 c.c. water + 7 gm. Na<sub>2</sub>CO<sub>3</sub> (added gradually in powder).

<sup>2</sup> *Per os* + 40 gm. of lard; greedily devoured.

an excretion of nitrogen in the urine of 3.82 gm. in six hours. The corresponding fasting rate would have been  $3.42 = (1.14 \times 3)$ . Higher nitrogen elimination is continued over four hours succeeding. The D:N ratios of the two hours preceding and the four hours following the glutamic acid period were approximately the same, and are the usual ratios, and therefore correspond to destruction of body protein. The ratio of the glutamic acid period, however, is 4.10:1. If it be assumed that the nitrogen from glutamic acid is removed during this period, then the nitrogen from body proteid would amount to  $3.82 - 0.47 = 3.35$  gm. The sugar derived from this would be  $3.35 \times 3.65 = 12.22$ . The extra sugar

belonging to glutamic acid would then be  $15.60 - 12.22 = 3.38$  gm. Therefore 5 gm. of glutamic acid containing 0.47 gm. of N administered subcutaneously to a dog causes an increase in sugar excretion of at least 3.38 gm. The ratio  $D:N = 3.38:0.47$  may be calculated to be  $D:N = 7.2:1$ .

A similar method of calculation was used in the work of the following day and also in the next experiment (see Table II). It should be noticed that this method of calculation allows for the *minimum* of sugar production. If perchance any of the glutamic acid escaped metabolism and appeared in the urine, the sugar production per gram of substance ingested would be larger than this method of calculation indicates.

Table II shows a typical case of phlorhizin glycosuria. The protein metabolism in the diabetic condition may be calculated as 320 per cent that of simple fasting. This rise in metabolism is not due to a condition of fever, for the animal had a normal temperature of  $38.4^{\circ}$  at the end of the experiment.

#### GENERAL DISCUSSION.

If one calculates the theoretical  $D:N$  ratios which may be obtained by the complete conversion into dextrose of various numbers of carbon atoms contained in glutamic acid, and also the quantity of dextrose which 10 gm. of glutamic acid would yield, one obtains the following table:

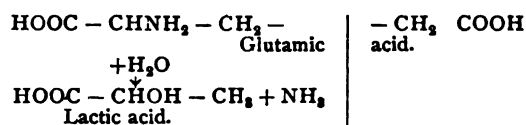
(Calculated.)		
	D:N.	10 gm. glutamic acid yield dextrose.
If 3 C atoms form dextrose . . . . .	7.26:1	6.90 gm.
If 4 C atoms form dextrose . . . . .	9.61:1	9.13 "
If 5 C atoms form dextrose . . . . .	12.53:1	11.90 "

The calculations of the physiological experiments show the following results:

(Experimental.)		
Dog.	D:N.	10 gm. glutamic acid yield extra dextrose.
V. Glutamic acid subcutaneously . . . . .	7.2:1	6.76 gm.
I. Glutamic acid subcutaneously . . . . .	9.2:1	8.75 "
V. Glutamic acid <i>per os</i> . . . . .	4.7:1	4.53 "
I. Glutamic acid <i>per os</i> . . . . .	7.1:1	6.73 "

If the lowest figure (4.7:1) be rejected, the other three results indicate the certain conversion of 3, and possibly 4, atoms of glutamic acid into dextrose.

If 3 atoms of carbon are converted into dextrose, as appears to be the most probable result, the following reaction would be indicated:



Two molecules of d-lactic acid may be completely converted into dextrose, as was shown by Mandel and Lusk.<sup>8</sup> The acetic acid group could be oxidized by itself. Neither this oxidation nor the oxidation of other fatty acids, however, would follow lines involving the formation of formic aldehyde, if the observations of Grube<sup>9</sup> are correct. Grube found that formic aldehyde perfused through the liver of a turtle is synthesized to glycogen. Since it has been demonstrated that sugar formation from fat is impossible, it is therefore improbable that formic aldehyde which may form sugar is produced from fat in the organism.

Investigations regarding the behavior of alanin in metabolism are now being carried on in this laboratory.

The writer acknowledges with thanks the co-operation of Mr. H. P. Mencken.

<sup>8</sup> MANDEL and LUSK: This journal, 1906, xvi, p. 129.

<sup>9</sup> GRUBE: PFLÜGER'S Archiv, 1908, cxxi, p. 636.

# THE TEMPERATURE COEFFICIENT OF THE VELOCITY OF NERVE CONDUCTION. (SECOND COMMUNICATION.)

BY CHARLES D. SNYDER.

[From the Department of Physiology, Johns Hopkins University, Baltimore.]

## INTRODUCTION.

IN my first communication<sup>1</sup> on this subject I expressed the idea that if a physical change were the fundamental process in any given physiological action, then the influence of temperature upon the velocity of the living process ought to be about the same as it is upon the velocity of the non-living process. For a long time the conduction of the nerve impulse has been thought of as a purely physical phenomenon. Indeed most of the important theories of nerve are based upon this assumption. But the particular physical process assumed has taken different forms, — from “polarization of molecules” to “shear along solid colloidal substance.” And so it occurred to me that we may be able to get some hint as to which of these physical processes is the right one to assume, by comparing the actual influence of temperature upon the velocity of nerve conduction with its influence upon the velocities of these various physical phenomena.

*The temperature coefficient of the nerve impulse as determined from observations of others.* — From the data available, however, it at once became evident that the velocity of the nerve impulse is affected by temperature much more like a chemical reaction than like any physical action which could obtain in the nerve.

This can be seen from the following table, which contains the constants of two of Nicolai's<sup>2</sup> experiments. In my original communication I based my calculations upon averages made up of the determinations of several of Nicolai's experiments. In the present table the calculation is based upon the measurements in single experiments. The table shows that the magnitude of the coefficient

<sup>1</sup> Archiv für Anatomie und Physiologie, Physiol. Abt., 1907, p. 113.

<sup>2</sup> NICOLAI: Archiv für die gesammte Physiologie, 1901, lxxv, p. 65.

is about the same whether individual or average constants are used as a basis.

The coefficient ( $Q_{10}$ ) is found by using the extra- and interpolation formula  $\left(\frac{k_1}{k_0}\right)^{\frac{10}{t_1-t_0}}$ , where  $k_1$  and  $k_0$  represent constants observed at the temperatures  $t_1$  and  $t_0$ .

Nicolai's work was upon the olfactory nerve of the pike. He made his measurements from photographic records of the oscillations of the capillary electrometer.

Date of experiment.	$t_1$ .	$t_0$ .	$k_1$ .	$k_0$ .	$\left(\frac{k_1}{k_0}\right)^{\frac{10}{t_1-t_0}}$ .
Nov. 6, 1900	19.25	6.5	18.6	7.5	2.03
	19.5	13.1	17.1	9.5	2.5
	19.25	4.8	18.6	6.9	2.0
	12.0	6.5	12.8	7.5	2.62
	12.0	4.8	12.8	6.9	2.36
Nov. 11, 1900	9.25	4.85	13.7	7.3	2.04
	9.2	3.6	13.7	6.4	3.85
	15.0	3.6	16.6	6.4	2.28
	18.6	9.25	19.5	13.7	1.52
	22.9	3.6	21.3	6.4	1.88
	25.0 <sup>1</sup>	4.85	22.2	7.3	1.72
<sup>1</sup> The constant at this temperature was determined from a second nerve of the same animal.					

Helmholtz had already remarked that "if a nerve [of the frog] were laid upon ice the time elapsing between the stimulation of the nerve and the mechanical action of the muscle is very remarkably prolonged, at times indeed as much as ten-fold."<sup>3</sup>

On page 345 of this same paper Helmholtz put down these constants for the velocity of the nerve impulse at room temperature:

1. At 20° C. a velocity of 29.1 m. per second.
2. At 20° C. a velocity of 25.1 m. per second.
3. At 21° C. a velocity of 26.9 m. per second.

<sup>3</sup> MÜLLER'S Archiv, 1850, p. 276. For this quotation see page 358.



It is from these determinations that we say the velocity of the nerve impulse in the frog is about 27 metres per second at 20° C. Now, if we assume that the nerve laid on ice is at 0° and that its velocity is decreased (as Helmholtz observed) ten-fold, then we may say its velocity at 0° is about 2.7 metres per second. The value of  $Q_{10}$  in this case is 3.16.

Von Miram<sup>4</sup> made determinations of the velocity of the impulse in frog's motor nerves at higher temperatures. The velocity in the ischiadicus at 35° he put at 65 metres per second; at 20°, 24 metres per second. The value of  $Q_{10}$  here is 1.95. From Helmholtz's casual observation and the more extended work of v. Miram one would be led to believe that the coefficient for the frog's nerve lay somewhere between 2 and 3.

The results of Gotch and Burch,<sup>5</sup> in their determination of the "critical interval" of nerve conduction at various temperatures, are of significance at this point. These authors found that, when the temperature was 4° C., two stimuli sent into a nerve at intervals less than 0.007" to 0.008" apart produced the effect of only one stimulus. If, however, the nerve was warmed up to 20° C., then this critical interval was lowered to 0.002" in duration. The coefficient of these constants is  $\left(\frac{0.0075}{0.002}\right)^{\frac{10}{20-4}}$ , or 2.3.

Thus, judging from the data in the literature and from a comparison of temperature coefficients, one is led to believe that the underlying cause of nerve conduction is not purely physical in its nature. Indeed, there is such an agreement in the character of this coefficient that in my first communication (in April, 1907) I concluded that the nerve impulse depended upon a chemical, and not upon a purely physical change.

#### NEW EXPERIMENTS.

It seemed desirable to carry out experiments to determine still more sharply, if possible, the velocity of the impulse at various temperatures. During the past fall and winter I have had excellent opportunity of doing this work, having at my disposal ample facilities and an abundance of good material.

<sup>4</sup> v. MIRAM: *Archiv für Anatomie und Physiologie, Physiol. Abt.*, 1905, p. 341.

<sup>5</sup> GOTCH and BURCH: *Journal of physiology*, 1899, xxiv, p. 410.

I wish here to take occasion to express my hearty thanks to Professor William H. Howell for extending to me the use of his laboratories for this work, and for his constant interest and valuable counsel, which lightened the routine and insured the prosecution of the work to a definitive outcome.

*Method.* — The method employed was that of Helmholtz, using Fick's pendulum myographion for the recording apparatus. A tuning-fork of about 200 double vibrations per second was used for measuring the time. The pendulum was made to swing with an amplitude great enough to move in space, at the point of the recording levers, with a velocity of about 11 mm. per 1/200 second. The instrument being constructed to compensate for temperature changes and for the slight vertical adjustments of the recording plate, this velocity was found to be practically constant. Every tracing of a muscle curve, therefore, was not accompanied by the time trace. By alignment the tip of the muscle lever could be made to occupy the same point in space as that of the tuning-fork in its test records, so that the velocity of the pendulum at any point of any one and of all the records was as nearly the same as possible. However, usually at least one time trace was taken for each experiment.

For stimulation a storage cell of about 2 volts was used. A coil of German silver wire being inserted at the positive pole, the current was then passed through the primary coil of an especially well-made inductorium. Both closing constant and opening induction shocks were used during the experiments, but mostly the latter.

Various devices for controlling the temperature of the nerve were employed. The arrangement proving to be most satisfactory, however, was somewhat as follows: A glass case,  $25 \times 20 \times 20$  cm., with vulcanized rubber bottom served as moist chamber for the entire muscle-nerve preparation. This, when closed, could be made practically air-tight, with the exception of a pin-hole opening in the bottom through which a thread could be passed connecting the muscle with the recording lever. Separate smaller chambers were provided for both muscle and nerve. The nerve chamber was made of vulcanized rubber in two parts, — an elongated box with closely fitting cover. One end of the box was pointed so as to admit its being brought close to the point where the nerve entered the muscle. Inserted into the bottom was a copper tank which extended the entire length of the box, and

which was provided with inlet and outlet tubes for circulating water. A delicate thermometer was inserted through a hole in the flat end of the box and laid along the length of the copper tank. Three pairs of platinum electrodes, held in place by binding-screws, lay across the thermometer tube at distances about 30 mm., 45 mm., and 65 mm. apart. The nerve could thus be laid along the length of the thermometer and at the same time on the electrodes, care being taken that the reading of the thermometer scale was not thus interfered with. The lid fitting down over nerve and thermometer, like the lid of a pill box, could be sealed at its joints with paraffine, thus making the chamber practically air-tight, excepting at the point where the nerve passed over to join the muscle on the outside. This opening could be easily modified with paraffine, so that it never was really larger than the diameter of the nerve. A long narrow window in the lid of the chamber enabled one to read the thermometer at any time, a mirror being used to reflect light onto the scale. The end of the chamber receiving the nerve was pointed. This permitted a very close approximation of the muscle chamber, and thus the use of all the length of the nerve excepting a very few millimetres.

The muscle chamber was entirely of glass, and consisted of an inner tube surrounded by an outer jacket, the latter being provided with tubules for circulating water. The inner tube was narrowed to a pin-hole opening at one end; the other was just large enough to receive the muscle and then permit of being closed with the knee joint and a cover of frog skin. The muscle was suspended by the femur bone, and hung in the muscle chamber, which was stood on end at right angles to the long axis of the nerve chamber. The angle where the two chambers met and where the smallest portion of the muscle and nerve were exposed was carefully covered over, and sealed with frog skin.

Thus, having separate chambers and separate water supplies for nerve and muscle, the temperature of one could be maintained or altered independently of the other.

In my experiments the temperature of the muscle was kept constant (at about  $20^{\circ}$ ), while that of the nerve was varied from about  $0^{\circ}$  to  $30^{\circ}$ . The separate chamber for the muscle was necessary to guard against temperature changes which would be caused by the circulating water of the nerve chamber.

Both nerve and muscle, by being enclosed in small spaces, were thus favored with a minimum amount of condensation or evapora-

tion changes. Frog skin was often used to wrap the nerve in, thus affording still more protection against concentration changes caused by variations in the humidity of the surrounding air. The large chamber enclosing the whole preparation would seem to be useless, but any one who has observed changes in atmospheric humidity due to air currents in a laboratory room will see at once that this was not an unnecessary precaution.

*Material.* — The material used was the ischiadicus nerve and the gastrocnemius and tibialis anticus muscles of species of edible frogs obtained from the local markets. From the largest of these, the "bull-frogs," it not infrequently happened that a length of nerve could be obtained measuring 70–75 mm.

*Calculating the velocity and the temperature coefficient.* — In calculating the velocity of an impulse from any given pair of contraction curves the usual method was employed. The following explanation of the formula used may be in order at this place:

Let  $D_s$  be the distance in millimetres between the far and the near pairs of electrodes; let  $k$  be the distance in millimetres between the ascending arms of the two muscle curves obtained by stimulating the nerve at the two different points touched by the electrodes, which are  $D_s$  millimetres apart; let  $t_s$  be the fraction of a second required by the pendulum to swing through 1 mm. of space at the point of the recording lever;  $t_s k$  would then be the difference in seconds of the latent period of the two muscle curves. If now we represent the velocity of the nervous impulse in metres per second by  $V$ , then we may say,  $V = \frac{D_s}{1000 t_s k}$ .

Usually  $k$  is the only variable in an experiment, in which case  $\frac{D_s}{1000 t_s}$  may be regarded as a constant,  $A$ , and our equation becomes

$V = \frac{A}{k}$ . The value of  $A$  of course would vary according to variations in the value of  $t_s$ , but as a matter of fact the arrangement of the apparatus was such that this factor remained practically constant throughout the investigation. Such small variations as did occur are noted along with the other data in the protocols.

The value of  $A$  also varies according to  $D_s$ , that is, according to the distance between the pairs of electrodes. Since this distance was varied from time to time, the value of  $A$  may be different for different experiments. Three distances were found convenient for the two pairs of electrodes, namely, 30, 45, and 65 mm. The values

of  $A$ , the other factors remaining constant, would be respectively ca, 65, 99, and 143.

The temperature coefficient,  $Q_{10}$ , is calculated by use of the extra- and interpolation formula referred to on page 180.

#### RESULTS.

In all, more than 60 experiments were performed, involving over 700 velocity determinations, which included, in round numbers, about 70 determinations, each at  $0^\circ$ ,  $5^\circ$ ,  $15^\circ$ ,  $25^\circ$ , and  $30^\circ$ , 130 at  $10^\circ$ , and 210 at about  $20^\circ$ . The results shown in the protocols printed below are typical of the whole series in so far as the muscle curves continued to be isometric.

I wish here to express my thanks to Professor Percy M. Dawson, who kindly undertook the laborious task of checking up my measurements.

*Explanation of the tables.* — In the following tables the headings to the columns are, for the most part, self-explanatory. Whenever the distance of the secondary coil was varied considerably during the experiment, this fact is noted in the column on the extreme left. Under the heading "Hour" are noted the hour and minute of the observations recorded in the respective places of the other columns. This is really an interesting and important item if one wishes to follow the history of the preparation, the approximate times of exposure to the various temperatures, the time allowed for the muscle and nerve to rest between stimuli, etc. Under " $t$ " or "Temperature" the temperature of the nerve (read from the thermometer in the nerve chamber, and upon which the nerve itself lay) is recorded for the corresponding observations of " $k$ " which appear in the column immediately adjoining. It will be remembered that " $k$ " represents the distance in millimetres between the two muscle curves resulting from stimulation of the nerve (at intervals of time one or two minutes apart and while remaining at a constant temperature) first at a point near the muscle, then at a point farther removed. Under the heading " $V$ " or "Velocity" is placed the velocities (in metres per second) of the nerve impulse as calculated from " $k$ " and the other data included in the factor  $A$  (see above). Under the heading " $Q_{10}$ " is placed the temperature coefficient of the velocity of the nervous impulse as determined from the values of " $k$ " (or " $V$ ") (and their corresponding temperatures) by the interpolation formula already mentioned.

**Experiment of Nov. 9, 1907.**—Frog's gastrocnemius muscle; *stimulation, make of constant current*; time of pendulum, 11.1 mm. per 1/200 second; electrodes, 44 mm. apart;  $A = 98.6$ .

Hour.	Temperature. degrees.	$\lambda$ . mm.	$V$ . m.	$Q_{10}$ .
2.52	13.5	5.0	19.6	
3.02	3.5	12.2	8.0	
3.07	3.0	12.8	7.7	
3.25	14.2	4.4	22.2	2.7
3.37	30.0	1.4	65.7	2.0
3.47	20.0	3.5	28.0	2.4
4.08	9.8	7.5	13.1	1.7
4.09	9.8	8.0	12.2	
4.25	1.0	15.9	6.1	2.6
4.33	1.0	18.7	5.2	

**Experiment of Nov. 13, 1907.**—Conditions the same, excepting electrodes were 29 mm. apart.

Hour.	Temperature. degrees.	$\lambda$ . mm.	$V$ . m.	$Q_{10}$ .
10.43	20.5	1.6	40.2	
11.08	1.0	11.1	5.8	
11.10	1.0	10.7	6.0	
11.37	18.5	1.7	37.9	2.7
12.29	19.0	2.3	28.5	
12.43	9.0	4.8	13.4	
1.13	10.0	4.7	13.7	
2.00	20.7	3.0	21.4	1.8

**Experiment of Nov. 23, 1907.**—Electrodes, 44.5 mm. apart; "glass tube" nerve chamber used instead of the nerve chamber described in the text; nerve lies on the thermometer; time, same; *stimulus, closing of constant current*.

Hour.	Temperature. degrees.	$\lambda$ . mm.	$V$ . m.	$Q_{10}$ .
3.26	21.5	5.25	18.7	
3.33	14.0	8.3	11.8	
3.42	8.0	26.0	3.7	3.2
4.12	15.5	8.7	11.2	
4.16	16.0	9.3	10.5	3.6
4.20	23.5	5.0	19.5	2.3
4.37	15.0	6.8	14.4	
4.41	9.0	12.0	8.1	2.6
4.48	7.0	21.6	4.5	

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**Experiment of Nov. 30, 1907.** — Nerve chamber described in text; *break induction shocks*; electrodes, 44.5 mm. apart; time, same.

Secondary coil.	Hour.	Temperature.	$\lambda$ .	$V$ .	$Q_{10}$ .
cm.		degrees.	mm.	m.	
14	11.34	8.1	10.5	9.3	
15	11.52	19.5	3.7	26.4	2.5
15	12.01	26.5	2.2	44.5	2.1
15	12.04	26.0	3.2	30.6	

**Experiment of Nov. 30, 1907.** — Preparation from the leg of same animal. Conditions all the same as above.

Secondary coil.	Hour.	Temperature.	$\lambda$ .	$V$ .	$Q_{10}$ .
cm.		degrees.	mm.	m.	
18	3.38	19.3	6.4	15.6	
18	3.45	27.0	3.0	32.6	2.7
18	3.56	Drying tetanus.			

**Experiment of Dec. 7, 1907.** — Electrodes, 29.5 mm. apart; time, 11.0 mm. per 1/200 second; load, 10 gm.; secondary coil, 25 cm. from primary. Nerve lying on thermometer.

Hour.	Temperature.	$\lambda$ .	$V$ .	$Q_{10}$ .
	degrees.	mm.	m.	
2.41	22.0	1.8	36.1	
3.00	8.8	5.3	12.3	
3.07	7.7	8.5	7.6	
3.29	20.5	1.8	36.1	2.8

**Experiment of Dec. 10, 1907.** — Load, 5 gm.; other conditions same as above.

Secondary coil.	Hour.	Temperature.	$\lambda$ .	$V$ .	$Q_{10}$ .
cm.		degrees.	mm.	m.	
15	3.22	19.5	3.0	21.6	
"	3.34	30.0	1.8	36.1	
"	3.36	30.0	1.8	36.1	1.8
"	3.44	19.8	3.8	17.0	
"	3.53	20.2	2.0	32.3	
"	4.13	10.0	5.2	12.5	2.6
12	4.37	6.0	7.6	8.5	2.6
"	4.46	16.5	2.5	26.0	3.0
"	5.03	25.0	1.5	43.3	2.0

**Experiment of Dec. 11, 1907.**—Electrodes, 44.3 mm. apart; load, 10 gm.; time, 11.0 mm. per 1/200 second; secondary coil at 15 cm.

Hour.	Temperature.	$\lambda$ .	$V$ .	$Q_{10}$
	degrees.	mm.	m.	
12.09	20.2	3.7	26.3	
1.08	2.0	16.0	6.1	2.2
1.21	10.6	7.0	13.9	2.7

**Experiment of Dec. 17, 1907.**—Electrodes, 44.3 mm. apart; secondary coil, 25–18 cm.; time, 10.6 mm. per 1/200 second; load, 10 gm.;  $A = 93.9$ .

Secondary coil.	Hour.	Temperature.	$\lambda$ .	$V$ .	$Q_{10}$
cm.		degrees.	mm.	m.	
25	11.38	19.6	4.8	19.5	
"	11.52	29.5	2.8	33.4	
"	11.53	29.3	2.1	44.6	
"	12.06	20.1	5.2	18.0	2.0
18	12.32	2.0	11.0	8.5	
"	12.34	2.0	12.0	7.8	
20	1.23	18.8	5.0	18.7	1.6
"	2.59	1.5	15.0	6.2	
"	3.01	1.5	15.0	6.2	
"	3.20	20.0	5.0	18.7	1.8
17	3.35	29.5	2.3	40.7	
"	3.38	29.5	1.4	67.1	
"	3.52	20.0	4.8	19.5	2.7
20	4.14	2.5	15.0	6.2	
"	4.16	2.5	14.9	6.3	
"	4.42	18.3	5.6	16.6	1.9
"	5.28	2.0	19.0	4.9	2.1

On the following day the same preparation:

20	11.53	18.0	4.8	19.5	
"	11.01	9.3	9.5	9.8	
"	11.03	8.4	10.0	9.4	
"	11.35	19.9	5.3	17.7	1.9



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**Experiment of Dec. 18, 1907.** — The gastrocnemius with its nerve was now prepared from the remaining leg of the frog, used on Dec. 17, 1907, with the following results; the conditions of the experiment remained the same:

Secondary coil.	Hour.	Temperature.	$\lambda$ .	$V$ .	$Q_{10}$
cm.		degrees.	mm.	m.	
20	2.15	19.7	4.6	20.4	2.0
"	2.16	19.7	4.7	20.0	
"	2.29	30.4	2.3	40.1	
"	2.31	30.4	3.4	27.6	
"	2.39	29.5	3.3		
"	2.53	19.8	4.5	20.8	1.9
"	2.55	19.8	5.0	18.7	
"	3.05	11.0	7.9	11.8	
"	3.09	10.5	8.6	10.9	
"	3.12	10.5	6.6	14.2	2.6
18	3.20	2.5	14.5	6.5	2.7
"	3.22	2.5	15.0	6.2	
"	3.24	2.5	14.0	6.7	
20	3.40	11.0	6.0	15.6	
18	3.44	11.0	6.0	15.6	2.3
"	3.45	11.0	7.0	13.4	
"	3.59	20.5	2.0	46.9	
"	4.02	19.0	4.2	22.3	
"	4.04	18.8	4.5	20.8	
"	4.16	31.2	3.0	31.2	1.5
"	4.18	31.0	3.3	38.4	
"	4.30	19.5	5.6	16.7	
"	4.33	18.5	6.4	14.6	

**Experiment of Dec. 21, 1907.** — Electrodes, 44.3 mm. apart; time and load same as on December 16; secondary coil at 15 cm.

Hour.	Temperature.	$\lambda$ .	$Q_{10}$
	degrees.	mm.	
2.35	20.2	3.7	2.4
2.47	31.0	1.5	
2.49	31.0	1.5	
3.08	27.0	4.0	

**Experiment of Dec. 23, 1907.**—Electrodes, 29.5 mm. apart; load, 10 gm.; time, 10.96 mm. = 1/200 second; value of  $A$ , 64.7; secondary coil at 25 cm. *First preparation* :

Hour.	Temperature. degree.	$\lambda$ . mm.	$V$ . m.	$Q_{10}$
10.49	20.2	2.5	25.9	
10.52	20.2	2.4	27.0	
11.09	30.8	0.9	72.0	
11.12	29.5	1.1	59.0	2.3
11.35	20.0	2.3	28.0	
11.45	10.5	4.3	15.1	
12.04	10.0	4.9	13.4	
12.23	19.6	2.2	29.4	2.1

*Second preparation* (from same frog) :

		$\lambda$ .	$V$ .	$Q_{10}$
3.48	[15.0	3.2	20.2]	
3.56	[15.0	2.9	22.3]	
3.59	14.8	2.9	22.3	
4.20	26.0	1.5	43.1	
4.25	25.0	2.1	30.8	
4.39	14.5	3.8	17.0	1.8
4.41	14.5	4.0	16.2	
4.51	4.0	11.5	5.6	
4.54	4.0	12.4	5.2	3.0
4.58	2.5	13.7	4.7	
4.60	2.5	14.5	4.4	
5.11	14.0	4.1	15.8	
5.13	14.0	3.9	16.6	3.0
5.24	26.0	2.7	24.0	
5.30	25.0	2.3	28.1	
5.45	16.0	3.5	18.5	1.6

**Experiment of Dec. 24, 1907.**—Electrodes, 44.3 mm. apart; load, 10 gm.; time, 10.96 mm. per 1/200 second; value of  $A$ , 94.85; secondary coil, 18–12 cm. *First preparation* :

Hour.	Temperature. degree	$\lambda$ . mm.	$V$ . m.	$Q_{10}$
12.59	[29.5	2.0	42.4]	
1.01	30.0	2.4	39.5	
2.45	29.5	2.7	35.1	
3.30	4.0	29.0	3.2	
3.33	4.0	48.0	2.0	2.6

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**Experiment of March 13, 1908.** — Electrodes, 44.0 mm. apart; load, 5 gm.; secondary coil, 20-18 cm.; muscle (gastrocnemius) kept at 20°; preparation from *R. escul.*; time, 11.1 mm. per 1/200 second.

Hour.	Temperature.	$k$ .	$V$ .	$Q_{10}$
	degrees.	mm.	m.	
11.37	24.0	2.0		
11.41	24.0	2.2	46.7	
12.06	11.5	8.7		2.8
12.27	11.5	6.8	12.7	
1.52	22.0	3.4		2.1
1.56	22.1	3.0	30.6	
2.20	11.1	7.6		2.2
2.26	11.3	7.5	13.0	

**Experiment of March 14, 1908.** — Preparation from same frog which was pithed yesterday. Conditions of experiment same as on March 13.

Hour.	Temperature.	$k$ .	$V$ .	$Q_{10}$
	degrees.	mm.	m.	
11.54	22.1	3.9	25.0	
12.28	11.2	11.0	8.9	2.9
12.33	11.2	14.0	7.0	
12.58	21.5	4.2	23.4	

**Experiment of March 16, 1908.** — Conditions the same.

Secondary coil.	Hour.	Temperature.	$k$ .	$V$ .	$Q_{10}$
cm.		degrees.	mm.	m.	
18	11.50	14.0	5.4	18.1	
"	12.30	22.4	3.0	32.6	2.0
"	12.55	13.0	5.9	16.6	
15	1.39	4.2	11.0	8.9	2.0
"	2.10	14.4	4.9	20.0	2.2
18	2.40	23.0	2.6	37.7	2.3
"	3.50	8.8	9.6	10.2	2.5

**Experiment of March 17, 1908.** — *First preparation*: conditions the same as above.

Secondary coil.	Hour.	Temperature.	$k$ .	$V$ .	$Q_{10}$
cm.		degrees.	mm.	m.	
18	11.14	22.1	3.3	29.7	
13	12.11	3.8	12.3	8.0	
11	12.18	3.5	13.6	7.2	
18	1.53	22.1	4.0	24.5	2.0
11	2.29	1.0	27.0	3.6	2.4

*Second preparation:* Fresh animal; secondary coil kept at 18 cm., muscle at 21°; other conditions the same.

4.05	11.5	5.7	17.2	
4.25	22.0	2.5	39.2	
4.30	22.0	2.7	36.3	
4.50	12.2	5.0	19.6	2.0
4.55	12.5	4.9	20.0	
5.10	3.0	12.6	7.8	
5.15	3.5	14.3	6.9	
5.30	12.7	5.4	18.2	2.7
5.35	13.2	5.1	19.2	
5.45	23.5	2.2	44.5	
5.50	22.5	3.0	32.7	2.1

**Experiment of March 18, 1908.** — Secondary coil, 15–11 cm.; gastrocnemius muscle kept at 20°; other conditions the same.

Secondary coil.	Hour.	Temperature.	$k$ .	$V$ .	$Q_{10}$
cm.		degrees.	mm.	m.	
13	2.16	21.6	2.5	39.2	
11	3.00	1.0	16.0	6.1	
11	3.22	1.8	15.3	6.4	
15	3.47	21.0	2.2	44.5	2.6

### DISCUSSION OF RESULTS.

From these experiments it is seen that the temperature coefficient of the velocity of nerve conduction in the sciatic of the frog lies for the most part between 2 and 3. This result agrees with the coefficient found from the constants determined by previous investigators, not only for motor, but also for sensory nerve, as in the olfactory nerve in the pike. This value (2–3) of the coefficient holds especially when one compares the constants obtained for any two successive changes of temperature.

If, however, one compares the velocity of the impulse observed at the same temperature but at different times during an experiment, one notices sometimes that the 2–3 value fails completely. For example, in the experiment of November 13, at first the velocity at 20° is about 40 metres per second; an hour later it is still the same, but at the end of nearly two hours the velocity at the same temperature is about 30, and after about three hours and twenty minutes it has been reduced to 21 metres per second.

On the other hand, in the experiment of December 17, the initial

velocity of 20 metres per second at  $20^{\circ}$  is maintained for five hours, and in spite of the many changes of temperature (a dozen ranging from  $1.5^{\circ}$  to  $30^{\circ}$ ) to which the nerve in the mean time had been subjected. This is also the case in the experiment of December 18 with one exception, — the constant for the hour, 3/59, where the velocity suddenly becomes more than twice what it was three minutes before at the same temperature. These cases are for  $20^{\circ}$ . At  $30^{\circ}$  the velocities are much more unstable.

That these "abnormal" constants are the actual velocities and not slips or irregularities of the recording apparatus is shown by the experiment for March 17 (second preparation) and also in the following one of March 18. Experiments which were carried out to determine the changes in velocity occurring in a nerve kept constantly at the same temperature, — at  $5^{\circ}$ ,  $10^{\circ}$ ,  $20^{\circ}$ , — however, indicate that a constant velocity may be maintained for hours, a slowing occurring only after two hours at  $20^{\circ}$ , and after even a longer period at  $10^{\circ}$ .

From this one is inclined to conclude that these unusual velocities which appear in the experiments are due to changes in the nerve substance caused by evaporation, when the nerve is warmed, and by condensation of water, when the nerve is cooled, — changes which are inevitable in a gaseous medium in spite of all precautions taken to guard against them.

But in the normal, living body we know that profound changes must take place in the substance of the nerve also, — changes which are involved in conditions referred to by such terms as "sluggishness," "alertness," "exaltation," "feeling fine," "feeling dull" or "stupid," "pink of condition," and "staleness." To my mind all these conditions might exhibit themselves, in large measure, as differences of *tempo*; i. e., the nerves carry stimuli much more quickly in one condition than in another. Furthermore, they are not necessarily permanent, but may change from one tempo to the other. It is indeed a matter of common experience that our bodies may be functioning in *andante* time at one hour and in *allegro* the next. That this difference is due to the nerve centres only, has yet to be proved.

Durig<sup>6</sup> has shown that the velocity of nerve conduction in frogs kept out of water decreases with the increase in loss of water from their bodies, and the peculiar condition of "kept" or "cooled"

<sup>6</sup> DURIG: Archiv für die gesammte Physiologie, 1902, xcii, p. 323.

### EXPLANATORY NOTE TO THE PLATE.

The record is from the experiment of March 17, 1908, second preparation; for protocol, see text.

The extreme left-hand column indicates the distance in centimetres of the secondary from the primary coil of the inductorium.

The middle column of figures indicates the hour and minute when the corresponding muscle curves were recorded.

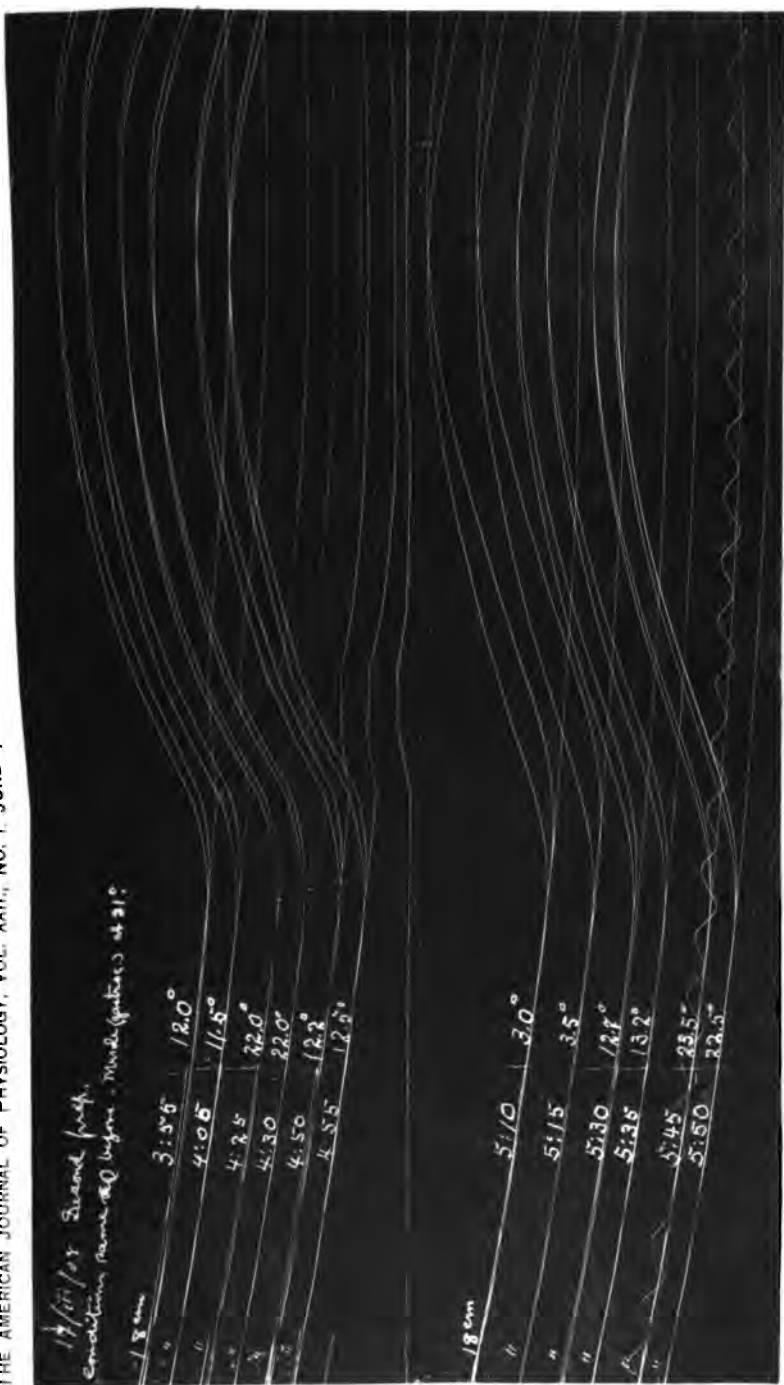
The column to the right of the "point of stimuli" marks indicates the temperature of the nerve as shown by the thermometer in the nerve chamber and upon which the nerve itself lay.

The time trace is from a tuning-fork vibrating 200 double swings a second. On the original sheet  $11.1 \text{ mm.} = 1/200 \text{ sec.}$

In this experiment the pairs of electrodes were 44 mm. apart, and the muscle, the gastrocnemius of the frog, was weighted with 5 gm., which hung unsupported.

The muscle was kept constantly at  $21^{\circ}$  by means of a surrounding water jacket.

The distance between the muscle curves (*i. e.*, *k*) is measured in every case by placing a ruler with 0.1 mm. divisions, so that it describes a cord to the base line with the 0 and 20 cm. marks on the edge of the ruler just touching the base line and the 10 cm. mark lying between the muscle curves.



C. D. SNYDER. VELOCITY OF NERVE CONDUCTION.





frog or muscle-nerve preparations is a matter of daily laboratory experience.

Now, in view of these facts, it seems to me that we are justified in regarding the different velocities of conduction in a nerve at the same temperature, not as irregularities of the apparatus, nor as manifestations of a diseased animal or of an abnormal and injured preparation, but rather as normal phenomena which must be included in any explanation of the more usual velocities observed.

It is quite conceivable that these observed differences in *tempo* of nerves may be due to changes in the physico-chemical structure of the nerve substance, — changes which occur not only in the excised and surviving organ but also in the normal living body. This change may be a change in the molecular aggregation of a single set of conducting substances, or a transference of the process from one set of molecules to another. It is not even necessary to assume that these different sets, or kinds, of conducting molecules are similar in any way at all excepting in their ability to conduct a stimulus by means of a chemical change. *But assuming that the chemical relations of one set are different from those of another, it would then become probable that their reaction times would also differ.*

This assumption that the conducting molecules of nerve are not all of one and the same constitution is in perfect accord with the more modern view taken concerning the chemical status of living protoplasm in general. If living matter of one kind is a "complex of molecules," there is no reason why nerve substance (say, of the axis cylinder) is not also a complex of molecules.

We may go one step further in our assumption (which I believe is also in the direction of the greater probability), and say that this complex is never a definite one; that is, *the functional life of the tissue does not depend upon one particular complex, but rather upon any one of certain possible complexes which obtain among the molecules of the conducting substance.*

*Now, if the conduction of nerve is a chemical change, the velocity of the time reaction may be of one magnitude for one complex and of another magnitude when another complex is brought into play.*

To illustrate, let us assume that the velocities produced when complex *A* is reacting at the respective temperatures, 0°, 10°, 20°, and 30°, are 4.5, 11.3, 28.3, and 70 metres per second; and that the velocities produced when complex *B* is reacting at the same

temperatures are 2.9, 7.2, 18, and 44 metres per second. The coefficients for both *A* and *B* are all 2.5. But suppose *A* reaches an end point somewhere between 20° and 30°, and that when the nerve is tested at 30° *B* only is reacting; then the determinations, *A* only acting up to 20°, would be 4.5, 11.3, 28.3, and 44; the coefficients, respectively, 2.5, 2.5, and 1.5.

Suppose, now, in another case it happened that *B* only was reacting at 0° and 30°, *A* only at 10° and 20°, — a case which may be conceived as being due to intermittent metabolic, or nutritional, exchanges; then our readings would be

Temp.	0°.	10°.	20°.	30°.
Velocities	2.9 ( <i>B</i> )	11.3 ( <i>A</i> )	28.3 ( <i>A</i> )	44 ( <i>B</i> )
$Q_{10}$	3.9		2.5	1.5

Now, these velocities and their coefficients are quite typical of the results of my investigations, not only on nerve, but also on the temperature velocities of other physiological processes. Usually the coefficients are 2–3 so long as the temperature is in the vicinity of 10°–20°, but more than 3 when 0° is approached and less than 2 when 30° is approached.

If the reader will turn to the protocol for December 23, second preparation, he will find an actual case in point. In round numbers (excepting the first constants at 15° and 25°) the values are, at about 5°, 15°, and 25° temperature, about 7, 18, and 25 metres per second. Now, these velocities may be explained as follows: Two complexes are present whose relative time reactions may be represented at the above temperatures as being, for *A*, 7, 18, and 45; for *B*, 4, 10, and 25. During part of the experiment (at 5° and 15°) *A* only is functioning. At 25° sometimes only *A*, sometimes only *B* is functioning, — the latter case being more common. And so we have the constants as shown above, — 7, 18, and 25, with their coefficients 2.5 and 1.4.

Such a system of reacting bodies cannot be wholly unknown to chemistry, and, happily, as this paper goes to press, an excellent illustration of the point has just come to my notice. In a system involving the chemical action of a catalyser upon both the optical isomers of a substance, or upon both an optically active substance and an optically inactive substance, at one and the same time, we may, by polarimetry, determine the relative velocities of both the

compounds undergoing catalysis. Bredig and Fajano<sup>7</sup> have been able to show "a well-marked difference between the decomposition velocities of *d*- and *l*-campho-carbonic dioxid under the influence of an optically active catalyser (nicotin), which," they go on to say, "reminds one forcibly of enzyme experiments such as those of Dakin. . . ." Now, Dakin's<sup>8</sup> experiments showed that the difference of velocity in the case of the enzyme, lipase, acting upon the optical isomers of asymmetric acid esters, became as great as 50-130 per cent. But these experiments had been preceded by experiments of Mackenzie and Harden,<sup>9</sup> who, working with the enzyme of pure cultures of *penicillium glaucum*, etc., had already pointed out that an enzyme might attack both of the optical isomers of a substrat, but with different velocities.

The very interesting point is that we have here not only actual examples of two or more chemical reactions going on at different velocities in the same system, — examples from the field of pure chemistry,<sup>10</sup> but also that we have examples from the field of enzyme reactions. This makes a natural bridge to the probability that the same thing occurs in living tissues and indeed in the functioning axis cylinder of nerve itself.

In the light of the hypothesis as outlined above, the more or less periodic occurrence of velocities which one may notice in my experiments seems to have some meaning. In the following table are listed the type constants of all my experiments, expressed in round numbers, and, when necessary, as estimates of probable constants for the intervals of temperature which I have selected for the sake of convenience. The 2-3 temperature coefficient of chemical action is the basis of the segregation of these constants into "orders." The members of an order in any one experiment are placed in the same horizontal line; the constants of different orders for the same temperature are placed in vertical columns.

The greatest number of orders of type constants occurring in a single experiment seems to be four, as seen in the experiment for December 17, 1907, an experiment whose observations were ex-

<sup>7</sup> BREDIG and FAJANO: *Berichte der deutschen chemischen Gesellschaft*, 1908, Jahrg. 41, p. 752.

<sup>8</sup> DAKIN: *Journal of physiology*, 1905, xxxii, p. 199.

<sup>9</sup> MACKENZIE and HARDEN: *Proceedings of the Chemical Society*, 1903, xix, p. 48.

<sup>10</sup> EMIL FISCHER really pointed the way to these experiments, see *Zeitschrift für physiologische Chemie*, 1893, xxvi, p. 83. (See BREDIG and FAJANO, *l. c.*)

Date of experiment.	Rate at 0°.	Rate at 10°.	Rate at 20°.	Rate at 30°.	Q <sub>10</sub> .
Nov. 9, 1907	..	(20)	(60)	..	3.0
	5	15	30	70	2-3
	..	..	(20)	(40)	2.0
Nov. 12, 1907	..	..	40	..	..
	..	15	30	..	2.0
	..	10	20	..	2.0
	1	..	..	20	..
Dec. 7, 1907	..	15	35	..	2.3
	..	10	(30)	..	3.0
Dec. 9, 1907	..	..	30	80	2.6
Dec. 10, 1907	..	..	40	(120)	3.0
	..	12	30	50	2.5
	2.5	..	20	..	3.0
Dec. 10, 1907 (Second preparation)	..	..	..	36	..
	..	..	30	..	..
	5	10	20	40	2.0
Dec. 11, 1907 (Second preparation)	..	15	(30)	..	2.0
	..	10	25	..	2.5
	2	..	..	30	2.5
Dec. 11, 1907	..	10	20	..	2.0
	2	5	..	..	2.5
Dec. 16, 1907	5	15	..	..	3.0
	..	10	20	..	2.0
	..	..	..	30	..
Dec. 17, 1907	5	..	(30)	(70)	2.5
	..	10	20	40	2.0
	..	..	10	30	3.0
	2.5	..	..	(20)	2.0
Dec. 18, 1907	5	15	45	..	3.0
	..	10	20	40	2.0
	..	..	..	30	2.0
	..	..	..	70	..
Dec. 23, 1907	..	15	30	60	2.0
	..	(10)	20	..	2.0
	2.5	..	..	..	..
Dec. 24, 1907	..	15	30	..	2.0
	..	..	20	40	2.0
	2.5	..	..	..	..
Dec. 24, 1907 (Second preparation)	..	20	..	..	..
	5	..	30	..	2.5
	..	..	..	40	..
Jan. 4, 1908	..	..	30	70	2.3
	..	..	25	55	2.2
	..	..	..	40	..
	..	..	..	30	..

*The Temperature Coefficient of Nerve Conduction.* 199

Date of experiment.	Rate at 0°.	Rate at 10°.	Rate at 20°.	Rate at 30°.	Q <sub>10</sub>
Jan. 9, 1908	..	10	20	..	2.0
Jan. 10, 1908	..	10	20	..	2.0
	..	..	10	..	..
Jan. 14, 1908	..	15	30	..	2.0
	..	..	20	40	2.0
	..	..	..	30	..
Jan. 17, 1908	..	15	30	..	2.0
	5	..	20	..	2.0
Jan. 20, 1908	10	20	40	..	2.0
	..	..	30	..	..
Jan. 22, 1908	..	..	40	..	..
	..	..	30	..	..
Jan. 23, 1908	5	15	30	..	2-3
Jan. 24, 1908	10	..	40	..	2.0
Mar. 2, 1908	..	20	..	..	..
	..	..	30	..	..
Mar. 3, 1908	..	15	30	..	2.0
	2.5	..	20	..	3.0
Mar. 4, 1908	10	..	40	..	2.0
	..	..	30	..	..
Mar. 5, 1908	10	..	..	..	..
	..	15	30	..	2.0
Mar. 13, 1908	..	15	40	..	2.7
	..	10	30	..	3.0
Mar. 16, 1908	..	15	30	..	2.0
	5	10	(20)	(40)	2.0
	..	(5)	..	30	2.5
Mar. 17, 1908	..	(15)	30	..	2.0
	5	..	20	..	2.0
	2.5	..	..	..	..
Mar. 17, 1908 (Second preparation)	5	15	40	..	3.0
	..	..	30	..	..
Mar. 18, 1908	..	(20)	40	..	2.0
	5	(15)	..	..	2.0
	..	..	30	..	..
	2.5	..	..	..	..
Mar. 19, 1908	10	25	..	..	2.5
	..	20	40	..	2.0
	..	..	30	..	..

tended over a period of twenty-four hours. Most of the experiments show only two orders of type constants. Those showing only one order do not always cover the briefest periods of experimentation.

It remains to be emphasized that the figures, as well as their arrangement, are largely artificial. The table is made to illustrate the hypothesis outlined above, and how that hypothesis may find application in the real findings of experiment.

Now, these orders, according to our hypothesis, represent different aggregates of molecules in the nerve substance which, singly or in conjunction with one another, may be thrown into action by a stimulus and thus produce the nerve impulse. The velocity of the nerve impulse depends, then, upon the *tempo*, or the *time reaction*, of the order or orders of molecular aggregates.

Without further comment it will be readily seen, from the periodic table, that the temperature coefficient of nerve conduction is 2-3 so long as the function is carried by any one of the orders of molecular aggregates, but may be more or less than this in case a transference of the process is effected during the experiment.

#### SUMMARY.

1. Exhaustive experiments, upon the sciatic nerve of frogs show that the temperature coefficient of the velocity of conduction, for the most part, lies between 2 and 3. This confirms the conclusion arrived at in a previous paper, where the data for the basis of the conclusion were derived from the work of other investigators.

2. In exceptional cases the coefficient is less than 2; more rarely still, greater than 3. These phenomena the writer assumes to be normal, and due to a difference in chemical time reaction of the conducting substances of the nerve.

3. The observed constants (of which there were over 700) admit of periodic arrangement in which the unusual or "freak" velocities take a normal and rational position.

4. This periodic variation of velocity at constant temperature is explained by assuming that more than one complex of molecules exist in the nerve substance which, singly or in combination, by undergoing chemical change, brings about the phenomenon of conduction. The several complexes, differing from one another (pos-

sibly only slightly) in their chemical relations, may thus also differ in their reaction times. Hence the phenomenon of variation in velocity of conduction of the same nerve at the same temperatures; hence, also, to some extent, variation in the *tempo* of sensory and motor reflexes,— of even “moods” and entire bodily activities.

5. Theories based upon the assumption that nerve conduction is a purely physical phenomenon are no longer tenable, because the temperature coefficients of the assumed physical phenomena are greatly less than for conduction of the nerve impulse itself. Any successful theory of nerve must take into account, or at least be in harmony with, the temperature factor.

## GALVANOTROPISM IN BACTERIA.

BY JAMES FRANCIS ABBOTT AND ANDREW CREAMORE LIFE.

THE response of bacteria to the constant current is a subject that has been very little investigated, the ordinary statement in the text-books being that they are not affected by a current of electricity too weak to destroy them. Since bacteria react so strikingly to chemotropic and other stimuli, and since the Protista in general show such an evident response to the galvanic stream, it has been rather remarkable that more definite information concerning the galvanotropism of bacteria has not been obtained. The difficulty has been, no doubt, one of technique. For the past three years the writers have been experimenting from time to time with various forms, and have obtained such constant results that it has seemed advisable to announce them.

### METHODS.

The forms experimented with have been the typhoid bacillus (*B. typhus*) from cultures obtained of the Health Department of St. Louis, *B. prodigiosus* from "wild" cultures obtained in the laboratory, *B. subtilis* from hay infusion, and a form of the *Termo* group obtained from a pea infusion.

Experiments with the hanging drop were uniformly unsuccessful, since the medium was drawn up by capillarity along whatever electrode could be devised. A method used by bacteriologists for studying anærobic bacteria under high powers by means of flattened capillary tubes was fairly successful; but the most satisfactory apparatus was the one which is figured below. To an ordinary slide there is cemented with balsam two parallel strips of cover glass to form a shallow trough between them. Within this trough are laid two pieces of very fine platinum wire, slightly flattened, and connecting with a well at either end of the slide,



which is filled with mercury. These wells are merely sections of small rubber hose cemented to the slide by balsam, and serve as convenient connections to introduce the current. When the cell is used, the central space is filled with the culture medium and covered with a thin cover glass. The distance between the tips of the two platinum electrodes is sufficiently small to be included in the field of a No. 7 Leitz objective. Experiments with various forms of brush "non-polarizable" electrodes appeared to afford no advantages over the bare platinum electrodes, and the latter have been preferred, because simpler. Both blunt and pointed electrodes were used.

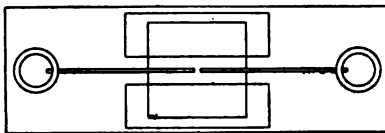


FIGURE 1.

In examining cultures of bacteria it was important to secure them as free as possible of the medium. A very small drop of the medium charged with bacteria was first transferred to a dish containing 2 or 3 c.c. of water.<sup>1</sup> A needle of this was introduced into the cell of the slide, filled with clean distilled water. The bacteria were thus washed twice. The fact of the purity of the water and the absence of electrolytes was evidenced by the enormous resistance of the cell.<sup>2</sup> The acid and alkaline modified media were made by adding a small amount of 1 per cent HCl and 1 per cent NaOH to neutral 10 per cent peptone beef gelatin (according to Frost).

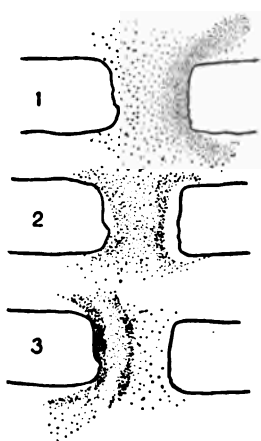
#### RESPONSE TO THE CURRENT.

When a cell is prepared as just described with a pure neutral culture of a motile form such as *Termo*, and a weak current of electricity passed through it, there is very quickly noticed a thinning of the bacteria in the neighborhood of one electrode and a corre-

<sup>1</sup> In the greater number of experiments water three times distilled against glass was used, but in the latter part of the work the water prepared for conductivity work in the laboratory of physical chemistry was employed.

<sup>2</sup> In the majority of the experiments great care was taken to measure the amount of current, resistance, etc. Use was made of a delicate D'Arsonval galvanometer of high resistance. A Daniel cell was used as a source of current, and a resistance box, together with the galvanometer, was always included in the circuit with the experiment slide. A marked reaction and definite reversible gathering may be obtained with so attenuated a current as is expressed by a resistance of 750,000 ohms and a current of 0.0000003178 ampere. In any case, of course, the current must be so weak that there shall be no electrolysis.

sponding aggregation about the other. When the current is reversed, the gatherings also change to the opposite pole. The following sketches will illustrate such an experiment.



EXPERIMENT XXX, June 8, 1906. Termo, liquefying gelatin. 1. Appearance after three minutes. 2. Current reversed, five minutes. 3. Same five minutes later.

A peculiar parabolic form of gathering is to be noted about the electrode. This is a very constant occurrence.

Similar results were obtained with all the motile forms tried, except when the culture was quite old.

#### EFFECT OF CHANGING THE MEDIUM.

Cultures from the same stock of *B. subtilis* were grown on both acid-modified and alkali-modified peptone gelatin, and it was found that in every case, if the culture was not too old to give any response at all, the bacteria from the one culture gave an opposite reaction to those from the other. This was repeated so often that there is no doubt of the result. In no case, however, was the effect so marked as to clear the field. There seem to be constantly a small per cent that are unaffected by the current. On the other hand, there is no counter gathering. It is always definitely at one pole or the other.

The following three sets of experiments, in which the organisms were all grown in rotation from the same strain, illustrate these conditions:

#### EXPERIMENT XLI. *B. subtilis*.

Date of culture.	Date of exp.	Medium.	Reaction.	Remarks.
Dec. 19, 1906.	Jan. 7, 1907.	Acid	- pole	Marked and definite.
Dec. 19, 1906.	Jan. 9, 1907.	Alk.	+ pole	Somewhat weaker.
Dec. 19, 1906.	Jan. 11, 1907.	Alk.	0	Sterilized by heat.

#### EXPERIMENT XLII. *B. subtilis*.

Jan. 14, 1907.	Jan. 19, 1907.	Acid	- pole	Rather weak reaction.
Jan. 14, 1907.	Jan. 19, 1907.	Alk.	+ pole	More marked.
Jan. 14, 1907.	Jan. 19, 1907.	Acid	0	Sterilized.

#### EXPERIMENT XLIII. *B. subtilis*.

Jan. 29, 1907.	Jan. 31, 1907.	Alk.	+ pole	Well marked.
Jan. 29, 1907.	Jan. 31, 1907.	Acid	- pole	Well marked.
Jan. 29, 1907.	Feb. 6, 1907.	Alk.	+ pole	Stronger than before.
Jan. 29, 1907.	Feb. 6, 1907.	Acid	- pole	Weaker than before.

In the next to the last instance there is apparently an exception to the statement made above, in that the older culture gave a more positive response. Almost always the reverse is true, and the explanation in this case is not obvious.

It will be seen that *B. subtilis* grown in an acid medium invariably gathers at the kathode, — that is, is negatively galvanotropic, — while if inoculations of the same parent cultures be grown in an alkaline medium the response is positively galvanotropic. This response, in practically all cases, is much more pronounced in young than in older cultures. If bacteria from a neutral culture be placed in a cell filled with  $n/100$  HCl or NaOH, they give the same kathodic or anodic response as those grown in acid and alkaline media. That this gathering at one or the other electrode is due to the motor response of the organism is proved by the fact that no non-motile bacteria (at least none observed by us) will give any response at all; furthermore, if *termo*, *subtilis*, or other motile forms be killed by heat, the reaction no longer takes place. The fact that old cultures react much less strongly than young ones is doubtless due to the same cause. That the gathering is not due to chemotropism is clear from the fact that the amount of current employed was far too small to bring about any electrolytic action, and the purity of the water used precluded a concentration of electrolytes at either pole. If the gatherings were due to the electrostatic condition of the bacteria themselves by virtue of which they were dragged up the potential gradient to either pole, the same should be equally true of non-motile organisms. But such we do not find to be the case. The conclusion, then, is that the motile bacteria are oriented by the galvanic current and swim to the electrodes, normally to the kathode under acid and neutral conditions, but reversing to the anode when immersed in alkali or grown in alkaline media.

The writers have tried to fix the bacteria to the cover while under stimulation in order to subsequently stain and discover the disposition of the flagella, but so far without success. Until something of the sort is done, it seems needless to speculate on the nature of the response in the organism itself, although *a priori* we might infer an inhibition of the flagella on one side and increasing stimulation on the other. Greeley's<sup>3</sup> tentative results with *Paramecium* under acid and alkaline conditions of environment would seem to be in harmony with those obtained with bacteria. The results here given

<sup>3</sup> GREELEY: Biological bulletin, 1904, vii, p. 316.

are purposely qualitative. Experiments are now being carried on with culture media made by adding definitely increasing amounts of acid and alkali.

#### SUMMARY.

1. *Termo*, *subtilis*, and typhus bacilli grown in a neutral medium when exposed to an extremely weak current form definite gatherings at the kathode.
2. Non-motile forms and motile forms that have been sterilized by heat show no such response.
3. By changing the pole the gathering may be repeatedly reversed.
4. Growing the above forms in acid-modified media or immersing the bacteria in weak HCl solution causes the bacteria to gather at the kathode or intensifies the normal kathode response. Growing them in alkali-modified media or immersing them in weak NaOH solution causes them to gather at the anode.

*Washington University.*





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# THE INFLUENCE OF INTERNAL HEMORRHAGE ON CHEMICAL CHANGES IN THE ORGANISM, WITH PARTICULAR REFERENCE TO PROTEIN CATABOLISM.<sup>1</sup>

BY FRED S. WEINGARTEN AND BURRILL B. CROHN.

[From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.]

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## I. INTRODUCTION.

CHANGES in the volume or in the character of the blood affect the body more or less promptly and in some cases most profoundly. A sudden shift of a large quantity of blood, as in shock,

<sup>1</sup> Every effort has been made, in the preparation of this paper, to present the data of these experiments in a manner closely analogous to that of the paper in

or a direct loss of much blood, as in ordinary external hemorrhage, will cause results varying in kind and degree with the quantity and the rapidity of the change as well as the part of the body involved. Our knowledge of the effects of hemorrhage, as well as of the resultant changes in the body and in the remaining blood, is limited. A host of investigators have observed the clinical changes after both accidental hemorrhage and blood-letting, and as many have been busy with studies of the morphology of the blood, its pathological changes in the various forms of anemia, the different degrees of importance of its components, its ability to regenerate after disease, etc. In recent years the defensive capacity of the blood has been particularly studied, and much attention has been paid to conditions affecting coagulation. As stated, however, in a previous paper from this laboratory, we had until recently comparatively little information on the chemical alterations resulting from large losses of blood.<sup>2</sup>

We have endeavored to increase our knowledge of the metabolic effects of internal hemorrhage. The series of experiments described in this paper is one of a group of researches (inaugurated in 1902 by Dr. Gies and now in progress in this laboratory) on the biochemical effects of change of volume of the circulating blood, and is the fifth of the number to be reported.<sup>3</sup> It was aimed especially to ascertain, in this series of experiments the effects of internal hemorrhage, under conditions similar to those of the earlier experiments in this laboratory on external hemorrhage, in which Dr. Gies was assisted by Drs. Hawk, White, and Hays, and Mr.

which the results of Dr. GIES's work with Drs. HAWK, WHITE, and HAYS, and Mr. SEIFERT on *external* hemorrhage were described by Dr. GIES (see HAWK and GIES: This journal, 1904, xi, p. 171). Direct comparison of the results of the two investigations is thus rendered very easy.

A preliminary report of this work appeared in the Proceedings of the Biological Section of the American Chemical Society in affiliation with Section C of the American Association for the Advancement of Science, New York meeting, December, 1906. WEINGARTEN: Science, 1907, xxv, p. 456.

<sup>2</sup> GIES: American medicine, 1904, viii, p. 185.

<sup>3</sup> GIES: *Ibid.*, p. 155; POSNER and GIES: Proceedings of the American Physiological Society: This journal, 1904, x, p. xxxi; HAWK and GIES: *Ibid.*, 1904, xi, p. 171; MEYER and GIES: Proceedings of the Society for Experimental Biology and Medicine, 1904, i, p. 44; WEINGARTEN: Proceedings of the Biological Section of the American Chemical Society, Science, 1907, xxv, p. 456.

Seifert.<sup>4</sup> Our experiments were accordingly made closely parallel to those described by Dr. Gies in the paper on external hemorrhage.

The problem in this research, intimately connected as it is with questions related to external hemorrhage, was viewed largely from the standpoint of the physician. We kept in mind particularly internal hemorrhages resulting from ectopic gestation, and from trauma causing laceration of intra-abdominal organs. Given such hemorrhage, and the surgical interference necessitated thereby, shall the blood be allowed to remain in the peritoneal cavity, and, if so, with what results to the organism? The observations made in our attempt to throw light upon these matters covered the physical as well as chemical changes in our subjects.

## II. PLAN AND METHODS OF THE EXPERIMENTS.

**Scope.** — We have been obliged, for lack of time and opportunity, to confine our attention almost wholly, in this study of the effects of internal hemorrhage, to the metabolism of nitrogen, sulphur, and phosphorus. The extent and number of our observations have doubtless been sufficient, however, to permit correct interpretation of our data.

**Precautions.** — Care in the selection and the treatment of our animals, regularity in the feeding and in the period terminations, and adequate control precautions were rigorously observed. It may be specially stated, in connection with this last point, that all our recorded analytic data represent averages of closely agreeing results obtained by us independently.

**Methods.** — Our metabolism work was conducted on two dogs by the various methods usually employed in, and previously described in papers from, this laboratory.<sup>5</sup>

**Animals and environment.** — The animals, two healthy, full-grown dogs, were selected with a view to their withstanding the strain of long confinement and numerous hemorrhages; nervous or delicate animals would have been useless. The dogs were kept in cages particularly well adapted for this work.<sup>6</sup>

<sup>4</sup> HAWK and GIES: This journal, 1904, xi, p. 171.

<sup>5</sup> GIES and collaborators: Biochemical researches, 1903, i, pp. 69 and 419 (Reprints 1 and 21); also HAWK and GIES: *Loc. cit.*

<sup>6</sup> GIES: This journal, 1905, xvii, p. 403.

**Food.** — The food consisted of a mixed diet of hashed lean beef, cracker meal, lard, bone ash, and water. The raw meat was preserved in a frozen condition.<sup>7</sup> The commercial cracker meal was kept dry in well-closed jars. Lard was obtained in small quantities at a time, so that a supply of fresh material would always be available. The bone ash consisted of the thoroughly incinerated, carbon-free, commercial product. Its advantages in such experiments, for regulation of the consistency of the fæces, have recently been indicated in some detail.<sup>8</sup> Ordinary tap water was taken.

In each of our experiments the dog was given his daily food at 10 A. M. The solids and water were well mixed. The mixture was always promptly eaten, even after the last hemorrhages of each experiment.

**Periods, weights.** — In the records of our experiments each day ended at 10 A. M., when the weight was taken just before giving the food. Our figures for weight represent the weight of the animal at the *end* of each day of record. We did not attempt to bring the dogs into nitrogenous equilibrium after each hemorrhage, but noted changes following each hemorrhage, whatever the plane of nutrition happened to be. Each of the dogs, however, was kept under preliminary observation and brought to constant body-weight as well as practical nitrogenous equilibrium before the beginning of the normal or prehemorrhage period. The data of this normal period of constant body weight and approximate nitrogenous equilibrium furnished the basis for estimating the changes after new conditions were effected.

**Anæsthesia.** — Internal hemorrhage in these experiments was brought about indirectly. Blood in known volume was taken from a femoral artery and directed without loss into the peritoneal cavity through a small abdominal incision. Each of our surgical operations for such production and control of internal hemorrhage was conducted on the animal while it was under *general* though very light anæsthesia inaugurated with ether-chloroform mixture.<sup>9</sup> We sought to duplicate, in this connection, the anæsthetic conditions that

<sup>7</sup> GIES: This journal, 1901, v, p. 235; Biochemical researches, 1903, i, p. 69 (Reprint 1).

<sup>8</sup> GIES: Proceedings of the American Physiological Society, 1903. This journal, 1904, x, p. xxii; also STEEL and GIES: This journal, 1907, xx, p. 343.

<sup>9</sup> The chloroform was added in order to shorten the preliminary struggle in opposition to the administration of the ether.

prevailed in the analogous experiments by Hawk and Gies, and postponed for future study, by Dr. Gies, the effects of internal hemorrhage in animals under the influence of a *local* anæsthetic.

Two questions naturally arose at the outset in this connection, — one regarding the probable metabolic influence of the anæsthetic, another relative to the possible effects of the necessary wounds in the hemorrhage operations. It was Dr. Gies's opinion, based upon his observations under similar conditions in this laboratory during the past half-dozen years (some of which were recorded in his paper with Dr. Hawk), that the measurable metabolic effects of light anæsthesia as planned for and conducted in these experiments could not be very decided, — that at most the metabolic effects would be relatively slight and transient. Furthermore, we were advised by Dr. Gies of the impossibility of perfectly checking the influence of general anæsthesia, whatever it might be, in work of this kind, because of the fact that the responsiveness of a dog necessarily changes during the progress of a hemorrhage experiment, *i. e.*, that "control" data obtained at the beginning, for example, would probably not apply strictly to the animal at the end of the experiment and *vice versa*. Anæsthesia itself would doubtless ordinarily change somewhat the responsiveness of an animal to repetitions of anæsthetic influences.

The weight of the evidence bearing on this question favors the conclusion that general anæsthesia, either with ether or with chloroform or with both, in normal dogs, causes increased excretion of nitrogenous matter in the urine, at least for a short time after the anæsthetic period. In an experiment by Hawk and Gies *light* ether anæsthesia<sup>10</sup> of a dog that had been subjected a week previously to external hemorrhage (2.93 per cent of body weight) caused slightly *decreased* excretion of nitrogenous and sulphur-containing products in the urine, but had no appreciable effect on phosphorus excretion. In a second dog under similar conditions practically the same result was obtained for nitrogenous excretion. Instead, however, of aiming to check the effects of the anæsthetic on each animal, we sought rather to conform so far as possible with the anæsthetic conditions of the experiments by Hawk and Gies, in the expectation that such a course would yield comparative data that could be interpreted without confusion. We think such has been the case.<sup>11</sup>

<sup>10</sup> Inaugurated with ether-chloroform mixture.

<sup>11</sup> Shortly after this paper had been prepared for publication, an excellent paper on this subject by Dr. HAWK appeared in the Journal of biological chem-

**Surgical operations.** — The probable metabolic effects of the purely surgical procedures, like those of anæsthesia, may not be ignored in work of this kind, yet such influences cannot be ascertained any more precisely than those exerted by anæsthesia in experiments of the kind described here. In the experiments by Hawk and Gies there appeared to be slightly increased catabolic effects after anæsthesia and operation combined, but the effects noted were comparatively minor. In this respect, as in the case of anæsthesia, we duplicated so far as possible the conditions in the experiments by Hawk and Gies, so that our results could be checked by theirs, and believed, furthermore, that the hemorrhage results would either be decided enough to bury the minor effects of anæsthesia or operation, or both, or would be so slight as to make impossible any conclusions one way or the other. These deductions seem to have been entirely warranted.

Our surgical operations were conducted under approved methods and were begun between 2 and 3 P.M.<sup>12</sup> The animal was brought quickly under general anæsthesia with a minimum amount of struggling. A small branch of a femoral artery was isolated, clamped, and a cannula inserted. The leg was then covered with a sterile cloth that was kept wet with warm physiological saline solution,

istry (1908, iv, p. 321). In continuation of the research suggested by Dr. GIES, and which Dr. GIES and Dr. HAWK accordingly began together in this laboratory, Dr. HAWK has reported effects of anæsthesia on the excretion of nitrogen in the urine of several dogs in nitrogenous equilibrium. Dr. HAWK states that increased excretion of nitrogen occurred in each case during the first twenty-four to forty-eight hours after the inauguration of anæsthesia. As a rule, the immediate increase was relatively slight. The "ultimate influence" of ether anæsthesia in six of the nine experiments (5 dogs) was an increase in the urinary output of nitrogen, but in three of the nine there was a decrease in the amount of excreted nitrogen. Fæcal elimination of nitrogen was not affected. Dr. HAWK concludes his paper as follows: "The data from our experiments would seem to indicate that, in all metabolism experiments upon dogs where the animal is subjected to ether anæsthesia, it is necessary to make check experiments to determine as accurately as possible the influence of such anæsthesia and to make the proper correction. At the same time our results further indicate that the possibility of determining what the exact influence of the narcosis was during the experiment proper, by a check test either before or after such experiment, is rather remote, inasmuch as the reaction of the animal to the anæsthetic will vary at the second administration of the anæsthetic from that observed as the result of the first narcosis."

<sup>12</sup> Operation at this hour occurred sufficiently long after feeding to minimize gastric discomfort from anæsthesia, but allowed time for recovery and rest before the next feeding.

while further preparations for the controlled hemorrhage were under way. Similar operations were performed upon each leg on several occasions. Consequently incision was made each time just above the place of previous entrance. The abdomen was opened with the usual precautions, and, after the first operation, in such a way as to avoid previous points of entrance. After about the fourth operation this was not an easy task. Similarly, it was increasingly difficult to find a satisfactory small artery in the leg, as adhesions, thrombi, and anastomoses obliterated anatomical landmarks.

With the abdomen open, blood was drawn from the artery and collected in a warm, sterile, graduated beaker. After the first operation we weighed the blood before pouring it into the peritoneal cavity, but increasing coagulation rates subsequently made it necessary for us to be satisfied with known volumes, from which the weights could be approximately calculated. At each hemorrhage we drew fairly rapidly a quantity of blood equal to from  $2\frac{1}{2}$  per cent to  $5\frac{1}{2}$  per cent of the weight of the dog as recorded at the beginning of the day of operation. As was the case in the work by Hawk and Gies, we experienced increasing difficulty in getting a free blood-flow toward the end of each operation, even when a relatively large vessel had been opened. This was due to the cumulative coagulability of the blood under the conditions of the hemorrhages and also to decreased blood-pressure. When the drawn blood clotted before it could be poured into the abdomen, as it did on several occasions, it was inserted *en masse* into the cavity and the serum poured after it. In two cases we tried to direct blood into the peritoneal cavity through a cannula introduced with a trocar. The first portion went in readily, but coagulation rapidly set in, and we introduced the remaining blood through the usual abdominal opening.

Although relatively large quantities of blood were drawn, the dogs were kept alive by our skilled anæsthetist, Mr. Christian Seifert, assistant in this laboratory.

The artery was ligated above and below the opening in it. The abdominal wall was sutured, layer by layer. Chromicized catgut was used for deep sutures, sterile thread for skin sutures. At first the wounds were covered with sterile pads kept in place by adhesive plaster. In later operations, however, a sterile dressing and colloidion were found sufficient, the dog taking excellent care of the wound after the dressing came off, as it always did within two days. Drainage was slight. All the wounds healed rapidly.

At no time was there any appreciable blood-loss, either in operating or transferring. At the beginning of each anæsthesia some urine was eliminated, but as we were prepared for such emergencies, it was always caught. The lengths of the operation periods were about the same as those in the experiments by Hawk and Gies.

The dogs recovered very promptly after the operations, except the last in each experiment. There was slight vomiting in several instances, but the animal promptly took the vomitus again without reluctance.

Ligating the arteries of the leg did not seem to cause either local or general reactions.<sup>13</sup> The vascular conditions were soon re-established, apparently, and at each operation we found scar tissue at the site of the previous one, with satisfactory anastomoses. There was no appreciable difference in the warmth of the legs.

**Collection of excreta.**—The urine was collected as voided, and was preserved in stoppered bottles with powdered thymol. The volume for each twenty-four hours was ascertained and recorded in the tables.

The addition of bone ash<sup>14</sup> to the diet increased the bulk of the fæcal matter, but made its discharge more frequent and regular. The fæces had the usual consistency and appearance of excrement passed by dogs on a diet containing bone. There was no diarrhœa, the fæces did not stick to the cage and could be easily dried and powdered. The fæces were promptly dried on a water-bath, and the daily quantity bottled after its weight, consistency, etc., had been noted. For analysis the fæces were powdered and sieved to eliminate hair. The bone ash made all this easy. At the end of each period, and before returning the dog to the cage, the latter was cleaned. The washings were analyzed. The analytic data for cage washings were added to the figures for urinary period totals.

**Analytic methods.**—Nitrogen, sulphur, and phosphorus were quantitatively determined in the ingredients of the food, and also in the urines and fæces. Only pure reagents were used. The urine was also examined clinically, from time to time, especially immediately after operations, for albumin and sugar.

All determinations of nitrogen were made by the Kjeldahl process.

<sup>13</sup> See also HAWK and GIES: *Loc. cit.*

<sup>14</sup> GIES: Proceedings of the American Physiological Society, 1903. This journal, 1904, x, p. xxii; also STEEL and GIES: *Ibid.*, 1907, xx, p. 343.



Oxidation was effected with concentrated sulphuric acid, aided by a little cupric sulphate. The acid decompositions were continued for an hour after the mixtures became colorless, in conformity with the custom in this laboratory.

Total sulphur and phosphorus were determined by the customary fusion processes. Specific gravity of urine and blood was determined approximately by a hydrometer.

### III. FIRST EXPERIMENT.

**Animal.**—The animal used in the first experiment was a mongrel bitch, plump and vigorous, weighing a little over 8 kilos.

**Diet.**—The character of the diet and the daily quantity were the same throughout the experiment. Data in this connection are given in Table I.

TABLE I.  
COMPOSITION OF THE DAILY DIET.<sup>1</sup>

	Hashed lean beef.	Cracker meal.	Lard.	Bone ash.	Water.
	gm.	gm.	gm.	gm.	c.c.
Daily amounts	128.0000	32.0000	24.0000	8.0000	320
Nitrogen . . .	4.7160	0.4960	0.0062	0.0024	....
Sulphur . . .	0.3644	0.0419	0.0072	0.0048	....
Phosphorus .	0.2880	0.0430	0.0206	1.4232	....
	Weight.	Nitrogen.	Sulphur.	Phosphorus.	
	gm.	gm.	gm.	m.	
Totals for each daily mixture	512	5.2206	0.4183	1.7748	
<sup>1</sup> Several lots of meat were used. These were found to vary so slightly in composition that, for purposes of computation, an average was taken of the various analyses (HAWK and GIES, <i>Loc. cit.</i> ). As the dogs were not kept in perfect nitrogenous equilibrium, this course did not materially affect our deductions.					

**Preparatory period.**—A preparatory period of twelve days, which brought the animal to the desired condition, was terminated on February 4 (1906) at 10 A. M. The dog then weighed 8.12 kilos. At this point the accumulation of analytic data was begun. The experiment continued without interruption until April 5, a period of sixty-one days, and terminated with the death of the dog on

the operating-table, as we were about to start a new hemorrhage period. The sixty-one days were divided into one period of normal conditions, with the animal in practical nitrogenous equilibrium, and five post-hemorrhagic periods.

**First period. Normal conditions. Maintenance of approximate nitrogenous equilibrium. Days, 1-5; February 4-8, 1908.** — Approximate equilibrium was maintained and a basis for subsequent comparisons afforded.

**Second period. First hemorrhage. Days, 6-21; February 9-24.** — On the sixth day we operated as previously described, trying at first to utilize a very small branch of a femoral artery. After many attempts we selected a somewhat larger one, the main saphenous branch, and inserted the cannula. A one-inch incision into the abdomen was made in the median line, just below the xiphoid. The blood was poured into a warm funnel connected with a rubber tube, and conveyed directly into the peritoneal cavity.

• *Schedule of operations.* — Anæsthesia started, 2.20 P. M. Operation started, 2.45. Cannula in artery, 3.24. Abdomen opened, 3.40. Blood drawn, 3.45. Blood in peritoneal cavity, 3.47. Abdomen closed, 4.10. Anæsthetic discontinued, 4.20. Leg closed and bandaged, 4.30. Dog up, 4.45.

*Blood.* — The quantity of blood transferred was 231 gm., an amount equal to 2.85 per cent of the dog's weight (8.09 kg.) at the beginning of the period.

**Third period. Second hemorrhage. Days, 22-33; February 25-March 8.** — Operation was started as before, but on the other leg. Preliminary difficulty with a very small arterial branch, as before, again necessitated the use of a larger one. Having cannulized the saphenous artery, a trocar and cannula were pushed through the left rectus, and wrapped about with cloth dipped in hot physiological saline solution. The drawn blood clotted before it could be completely poured through the cannula. Thereupon the usual abdominal incision was made, this time through the left rectus, so as to include the opening made by the trocar. The clot and serum were then passed into the peritoneal cavity, which was closed without further incident. The dog vomited slightly during the night, and some of this was admixed with a fraction of the urine. On filtering, however, a fair separation was made, and the vomitus added to the diet for that day.

*Schedule of operations.* — Anæsthesia started, 2.45 P. M. Operation started, 3.14. Artery exposed, 3.21. Trocar in abdomen, 3.53. Abdomen opened, 4.00. Blood in abdomen, 4.20. Abdomen closed, 4.55. Anæsthetic discontinued, 5.00. Leg closed, 5.18. Dog up, 5.25.

*Blood.* — The quantity of blood transferred was 245 gm., an amount equal to about 3.15 per cent of the weight of the animal at the commencement of the period.

**Fourth period. Third hemorrhage. Days, 34-42; March 9-17.** — Operation was started as usual. A femoral artery was entered at a point as far distally as possible. When the blood had been drawn, its coagulation was so rapid that our usual small abdominal incision was insufficient and had to be lengthened about half an inch to admit the clots, which were pressed through the opening and followed by the serum. The animal vomited slightly toward evening, but the ejected material was successfully caught. It was promptly eaten by the animal. The extreme hemorrhage was the feature of this operation. Blood was drawn until the animal showed extreme cardiac distress. The dog recovered promptly, however, and was lively and well during the entire period.

*Schedule of operations.* — Anæsthesia started, 2.15 P. M. Operation started, 2.45. Artery exposed, 3.00. Abdomen opened, 3.02. Anæsthetic discontinued, 3.05. Blood drawn, 3.07. Blood in abdomen, 3.15. Abdomen closed, 3.42. Leg closed, 4.00. Dog up, 4.10.

*Blood.* — The quantity of blood transferred was 340 grams, an amount equal to about 4.31 per cent of the weight of the animal at the opening of the period.

**Fifth period. Fourth hemorrhage. Days, 43-52; March 18-27.** — Operation was started by exposing a femoral artery as far distally as possible. The abdomen was opened on the right side by a Kammerer incision. On account of scars and adhesions the incision had to be made an inch and a half long. Extreme care had to be taken in closing the abdomen on account of the before-mentioned fibrosis. The deep layers were closed with fine interrupted catgut sutures, the fascia and skin with silk. A three-inch adhesive band retaining a sterile pad was put about the body. The plan had been to exceed the amount of the previous hemorrhage, but heart beat and respiration, as well as blood-pressure, were at such a low ebb that we were unable to do so. Rapid blood-clotting was a feature. In spite of the conditions that prevailed, recovery was prompt.

*Schedule of operations.* — Anæsthesia, 2.18 P. M. Leg opened, 2.38. Artery cannulized, 2.47. Abdomen opened, 3.06. Blood drawn, 3.12. Blood into abdomen, 3.25. Abdomen closed, 4.26. Leg closed, 4.40. Anæsthetic discontinued, 4.20. Dog up, 4.30.

*Blood.* — The quantity of blood transferred was 260 grams, an amount equal to about 3.5 per cent of the dog's weight at the opening of the period.

While the dog was bright she was very much weakened, and recovery from the immediate effects of operation was less prompt than before. All food was as promptly eaten as at first, however.

**Sixth period. Fifth hemorrhage. Days, 53-61; March 28-April 5.** — Operation was conducted as usual. The femoral artery was entered as far distally as possible, and the abdomen was opened in the same way as for the previous hemorrhage but on the opposite side. Owing to specially rapid clotting, the blood had to be drawn and introduced intraperitoneally in three separate portions. Blood was drawn to the limit of the dog's endurance.

**Schedule of operations.** — Anæsthesia started, 2 P. M. Leg opened, 2.21. Artery cannulized, 2.30. Abdomen opened, 2.34. Blood drawn and into abdomen, 2.38 to 2.41, 2.41 to 2.52, 2.52 to 2.57. Abdomen closed, 3.20. Leg closed, 3.30. Anæsthetic discontinued, 3.50. Dog up, 4.00.

**Blood.** — The quantity of blood transferred was 250 grams, an amount equal approximately to 3.54 per cent of the dog's weight at the opening of the period.

The dog showed seriously the drain upon the system. She seemed to be recovering gradually throughout the period (days 53-61), but was on a very low plane of vitality even at the end, as evidenced by sudden death, probably from shock just before bleeding at the close of the sixth operation, which was conducted in the usual way on the sixty-second day.

**Autopsy.** — There was no free fluid in the abdomen. The organs presented an appearance of extreme anæmia. Heart and lungs were normal. In life the gums and tongue of the animal had been pale after the third operation. The abdominal viscera were free from adhesions. The omentum was not discolored.

**Supplementary data. Periods I-VI.** — The room temperature averaged about 21° C. Respiration and blood-pressure during the operations varied with the amount of blood drawn, the rapidity of the hemorrhage, and the condition of the animal.<sup>16</sup> In the later operations cardiac and respiratory distress became evident much sooner than in the earlier ones. There was always a slight increase of pulse rate for about twenty-four hours after the operation, with irregular action for about the same time after the last operation. Simultaneously there was increased respiration. Pulse and respiration rates were practically normal after about forty-eight hours. Temperature was slightly elevated at the beginning of each hemorrhage period. The secretion of saliva during the anæsthesia periods was relatively abundant at first, as is usual during light

<sup>16</sup> DAWSON: This journal, 1900, iv, p. 6.

ether anæsthesia, but with increasing anæmia salivary secretion decreased. As a rule, the urine was acid to litmus. Immediately after hemorrhage (anæsthesia?) the urine was amphoteric to litmus. It never became markedly alkaline to that indicator. There was very little edema connected with any of the operations; the local reactions were very slight, and the dog seemed to suffer no particular discomfort because of them. With each operation the condition of anæmia became more evident.

Neither albumin nor sugar was present in abnormal quantity in any of the urines.

**Analytic results.** — Summaries of the results of the first experiment are given in Tables II–XI.

**Discussion of results.** *Decline in body weight.* — In the experiments by Hawk and Gies there was, naturally, a fall in body weight after each external hemorrhage, owing very largely to the actual abstraction of material from the body. The total loss of weight at the end of eighty-five days, after five hemorrhages, was 25 per cent. Our dog lost weight constantly and fairly steadily. There was a slight loss during the ante-operative period. The decrease was chiefly post-operative, however. There were appreciable gains at the ends of several periods upon the figures of the first few days of the corresponding periods. The total loss in weight, after five internal hemorrhages, was 11 per cent, or less than half of that noted by Hawk and Gies after five *external* hemorrhages. Much of the loss in our experiments, as in those by Hawk and Gies, was doubtless due to the direct effects of the anæsthetic.

*Fluctuations in the volume of urine.* — In general, as was noted by Hawk and Gies, for external hemorrhage, there was decreased elimination of urine on the day of operation, but increased excretion on the second or third day afterwards (Table III). The day of maximum urine output varied from the second to the ninth day. In the sixth period, however, there was an apparent exception, the output of urine on the day of operation having been the highest for the period, *i. e.*, 330 c.c. in comparison with 235 c.c. on the preceding day. On the day before the operation, however, there was an accidentally low elimination. A fraction of the urine belonging to that day was undoubtedly excreted with the volume passed on the day of operation (fifty-third). The average daily elimination of urine generally decreased after each hemorrhage except the last.

TABLE II.  
SOME OF THE DAILY RECORDS OF THE FIRST METABOLISM EXPERIMENT.<sup>1</sup>

First period. — Maintenance of approximate nitrogenous equilibrium. Feb. 4-8, 1906.							
DAY.	Body weight.	URINE.					Fæces dry.
No. in the experiment.		Volume.		Sp. gr.	Nitrogen.		
		Daily.	Period av. to date.		Daily.	Period av. to date.	
	kilos.	c.c.	c.c.		gm.	gm.	gm.
1	8.12	310	....	1.016	4.87	....	9
2	8.10	300	305	1.016	4.69	4.78	18
3	8.07	320	310	1.017	5.30	4.95	22
4	8.06	310	310	1.016	4.73	4.90	13
5	8.09	300	308	1.014	4.42	4.80	8
Second period. — Effects of operation and first hemorrhage (2.85 per cent). February 9-25, 1906.							
6	8.00	245	....	1.019	5.14	....	12
7	7.95	270	257	1.015	4.44	4.79	6
8	7.96	295	270	1.017	5.50	5.03	24
9	7.95	240	262	1.024	5.42	5.13	25
10	7.96	340	278	1.019	7.02	5.50	10
11	7.88	270	277	1.019	5.68	5.60	12
12	7.82	270	276	1.022	6.09	5.67	12
13	7.82	262	274	1.020	5.35	5.63	22
14	7.84	288	275	1.016	4.71	5.53	2
15	7.82	290	277	1.020	5.63	5.54	18
16	7.80	312	280	1.015	4.53	5.45	18
17	7.72	330	284	1.018	5.77	5.44	12
18	7.77	320	287	1.015	4.75	5.39	14
19	7.79	307	289	1.016	4.54	5.33	17
20	7.81	285	288	1.012	3.38	5.20	16
21	7.77	350	292	1.018	5.97	5.25	8
Third period. — Effects of operation and second hemorrhage (3.15 per cent). February 25-March 8, 1906.							
22	7.54	200	....	1.018	3.05	....	5
23	7.50	360	280	1.014	5.56	4.30	18
24	7.56	255	271	1.020	5.38	5.00	19
25	7.57	270	271	1.020	5.85	4.96	8
26	7.60	270	271	1.022	5.61	5.09	23
27	7.62	276	271	1.020	5.61	5.18	16
28	7.63	307	277	1.019	5.80	5.27	10
29	7.61	335	284	1.018	5.84	5.34	12
30	7.62	310	287	1.011	3.28	5.11	15
31	7.59	350	293	1.017	5.60	5.16	16
32	7.62	250	289	1.018	4.66	5.11	20
33	7.58	330	293	1.017	5.47	5.14	13
1 Facts regarding the daily food are given in Table I.							

TABLE II (continued).

Fourth period. — Effects of operation and third hemorrhage (4.31 per cent). March 7-17, 1906.							
DAY.	Body weight.	URINE.					Fæces dry.
No. in the experiment.		Volume.		Sp. gr.	Nitrogen.		
		Daily.	Period av. to date.		Daily.	Period av. to date.	
	kilos.	c.c.	c.c.		gm.	gm.	gm.
34	7.39	235	....	1.026	4.55	....	5
35	7.48	352	293	1.017	6.28	5.41	10
36	7.38	245	277	1.014	3.27	4.70	25
37	7.39	270	275	1.022	6.13	5.07	19
38	7.43	250	270	1.020	3.23	4.69	16
39	7.46	225	262	1.015	3.25	3.91	7
40	7.48	330	272	1.017	5.47	4.60	12
41	7.51	295	275	1.018	4.66	4.61	12
42	7.50	290	277	1.016	4.58	4.60	14

Fifth period. — Effects of operation and fourth hemorrhage (3.57 per cent). March 18-27, 1906.							
43	7.35	210	....	1.020	2.82	....	11
44	7.35	310	260	1.022	7.32	5.07	12
45	7.26	275	265	1.014	3.85	4.66	21
46	7.35	225	255	1.018	4.21	4.55	15
47	7.35	270	258	1.020	5.20	4.68	13
48	7.36	255	257	1.017	4.65	4.68	12
49	7.48	264	258	1.018	4.85	4.76	11
50	7.38	316	265	1.020	5.90	4.85	4
51	7.37	325	272	1.017	4.91	4.86	8
52	7.40	235	268	1.014	3.33	4.71	8

Sixth period. — Effects of operation and fifth hemorrhage (3.54 per cent). March 28-April 5, 1906.							
53	7.22	330	....	1.025	7.12	....	29
54	7.20	307	318	1.020	3.88	5.50	14
55	7.10	290	309	1.018	5.14	5.38	19
56	7.17	260	296	1.018	4.93	5.27	11
57	7.18	245	286	1.020	4.67	5.15	16
58	7.19	267	283	1.016	3.45	4.87	20
59	7.20	287	283	1.018	4.95	4.88	15
60	7.21	305	286	1.017	4.70	4.85	8
61	7.21	305	288	1.017	4.70	4.84	16

TABLE III.

TOTAL URINE VOLUMES ON THE EARLIER DAYS OF THE PERIODS.

Conditions.	Period no.	First day.	Second day.	Third day.	Fourth day.
Normal . . . . .	I	310	300	<del>320</del>	310
First hemorrhage . .	II	245	270	<del>295</del>	240
Second hemorrhage .	III	200	<del>360</del>	255	270
Third hemorrhage .	IV	235	<del>352</del>	245	270
Fourth hemorrhage .	V	210	<del>310</del>	275	225
Fifth hemorrhage .	VI	<del>330</del>	307	290	260

TABLE IV.

ANALYTIC TOTALS AND AVERAGES FOR NITROGEN IN EACH PERIOD.

Period number . . . . .	I 5	II 16	III 12	IV 9	V 10	VI 9
Days in the period . . .						
Conditions of the period .	Normal.	{ First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.
Food . . . . .	gm. 26.10	gm. 83.52	gm. 62.64	gm. 46.98	gm. 52.20	gm. 46.98
Excreta . . . . .	25.53	89.43	64.94	43.06	50.45	46.50
Total balance . . . . .	+ 0.57	- 5.91	- 2.30	+ 3.92	+ 1.75	+ 0.48
Average daily balance .	+ 0.11	- 0.37	- 0.19	+ 0.43	+ 0.17	+ 0.05

TABLE V.

NITROGEN IN THE URINE, PER PERIOD.

Period number . . . . .	I 5	II 16	III 12	IV 9	V 10	VI 9
Days in the period . . .						
Conditions of the period .	Normal.	{ First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.
Daily average . . . . .	gm. 4.80	gm. 5.25	gm. 5.14	gm. 4.60	gm. 4.71	gm. 4.84
Average, first three days	4.95	5.03	5.00	4.70	4.66	5.38
Average, last three days	4.82	4.63	5.24	4.90	4.71	4.78



TABLE VI.  
NITROGEN IN THE FÆCES, PER PERIOD.

Period number . . . . .	I	II	III	IV	V	VI
Days in the period . . . .	5	16	12	9	10	9
Conditions of the period .	Normal.	{ First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.
Total . . . . .	1.50	4.55	3.19	1.37	3.37	2.92
Daily average . . . . .	0.30	0.28	0.26	0.15	0.33	0.32

TABLE VII.  
QUANTITATIVE FÆCAL ELIMINATIONS, PER PERIOD.

Period number . . . . .	I	II	III	IV	V	VI
Days in the period . . . .	5	16	12	9	10	9
Conditions of the period .	Normal.	{ First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.
Dry weight: period total	70	228	175	120	115	148
" " daily average	14	14	15	13	12	16

TABLE VIII.  
SUMMARY OF ANALYTIC DATA FOR TOTAL SULPHUR AND PHOSPHORUS METABOLISM.

Period.		SULPHUR.				PHOSPHORUS.			
		Total.		Balance.		Total.		Balance.	
No.	Conditions.	In-gested.	Ex-creted.	Total.	Daily Average.	In-gested.	Ex-creted.	Total.	Daily Average.
I	Normal	gm. 2.09	gm. 1.71	+0.38	+0.0760	gm. 8.87	gm. 8.34	+0.57	+0.1140
II	First hemor.	6.69	5.27	+1.42	+0.0887	28.39	21.71	+6.68	+0.4175
III	Second "	5.01	4.44	+0.57	+0.0475	21.29	24.12	-2.83	-0.2358
IV	Third "	3.76	3.16	+0.60	+0.0666	15.97	13.77	+2.20	+0.2440
V	Fourth "	4.18	2.86	+1.32	+0.1320	17.74	12.57	+5.17	+0.5170
VI	Fifth "	3.76	3.64	+0.12	+0.0133	15.97	19.20	-3.23	-0.3580

*Specific gravity of the urine.* — This varied from 1.011 to 1.026. It generally increased on the day of hemorrhage, contrary to what

TABLE IX.  
QUANTITATIVE SULPHUR EXCRETION, PER PERIOD.

Period number . . . . .	I 5	II 16	III 12	IV 9	V 10	VI 9
Days in the period . . . . .						
Conditions of the period . . . . .	Normal.	{ First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.
Urine . . . . .	gm. 1.43	gm. 4.38	gm. 3.76	gm. 2.67	gm. 2.31	gm. 3.00
Fæces . . . . .	0.284	0.89	0.686	0.493	0.545	0.639
DAILY AVERAGES.						
Urine . . . . .	0.28	0.27	0.31	0.29	0.23	0.33
Fæces . . . . .	0.056	0.055	0.057	0.054	0.054	0.071

TABLE X.  
QUANTITATIVE PHOSPHORUS EXCRETION, PER PERIOD.

Period number . . . . .	I 5	II 16	III 12	IV 9	V 10	VI 9
Days in the period . . . . .						
Conditions of the period . . . . .	Normal.	{ First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.
Urine . . . . .	gm. 1.04	gm. 4.14	gm. 3.12	gm. 2.49	gm. 2.48	gm. 2.60
Fæces . . . . .	7.30	17.57	20.99	13.89	10.99	16.60
DAILY AVERAGES.						
Urine . . . . .	0.20	0.25	0.26	0.27	0.24	0.28
Fæces . . . . .	1.46	1.09	1.74	1.54	1.09	1.84

our predecessors found for external hemorrhage. In the third period there was no change in this respect on the day of operation, although the fall in urinary volume was very sharp.<sup>17</sup> While fluctu-

<sup>17</sup> Practically all of the volume for the day was eliminated before the operation, however.

ating considerably, specific gravity was generally lowest at the ends of hemorrhage periods. The maximum figure for specific gravity was recorded on the fourth day in the second period, on the fifth day in the third period, on the first day in the fourth period, on the second day in the fifth period, and on the first day in the sixth period. The changes were not regular enough to warrant any general deduction regarding their significance.

*Nitrogen catabolism.* — Elimination of urinary nitrogen was increased during the first few days of each hemorrhage period. After the first and last hemorrhages such increases occurred on the first

TABLE XI.

SUMMARY OF PERIOD, AVERAGE DAILY BALANCES FOR NITROGEN, SULPHUR, AND PHOSPHORUS.

Period number . . . . .	I	II	III	IV	V	VI
Conditions of the period . .	Normal.	{ First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.
Nitrogen . . . . .	gm. +0.110	gm. -0.370	gm. -0.190	gm. +0.430	gm. +0.170	gm. +0.050
Sulphur . . . . .	+0.076	+0.089	+0.048	+0.067	+0.132	+0.013
Phosphorus . . . . .	+0.114	+0.418	-0.236	+0.244	+0.517	-0.358

day; after the remaining hemorrhages, increased elimination of urinary nitrogen took place later in the periods. Individual figures for urinary nitrogen were lower, as a rule, in the last three periods than in the first three. The average daily urinary elimination of nitrogen was higher by the end of the first two hemorrhage periods than at their beginnings. The reverse was true of the similar daily averages for the last three hemorrhage periods. The highest final daily average for a period was recorded after the first hemorrhage; the lowest, after the third hemorrhage.

Comparison of the daily average amount of nitrogen in the urine of the last three days of a period with that of the first three days of the hemorrhage period following shows an increase after hemorrhage in three of the five periods (first, second, and fifth). The daily average amount of urinary nitrogen for the whole of the second period (after the first hemorrhage) was *higher* than that for either the first or last three days of that period. In the fourth

period (after the third hemorrhage), on the other hand, the general average was *lower* than the averages for the first and last three days.

The daily average amount of faecal nitrogen per period shows a regular decrease to the fourth period, whereas the daily average amount of urinary nitrogen per period rose in the second period before decreasing regularly to the fourth period. But whereas the average daily excretion of urinary nitrogen per period was practically "normal" after the fourth period, the average elimination of nitrogen per period in the faeces was greatest in the fifth and sixth periods.

On comparing the total amounts of nitrogen ingested with those excreted it is evident that there were minus balances after the first and second hemorrhages but plus balances thereafter (Table IV).

These results suggest that after the first two hemorrhages the nitrogenous matter in the transferred blood was lost from the system in large part, either directly or indirectly, — the animal's nitrogenous surplus was apparently sufficient to meet immediate needs. It might also be inferred, however, from the results in Table IV, that the animal's nitrogenous surplus had been exhausted after the second hemorrhage, for after each of the last three hemorrhages practically all of the nitrogenous matter of the transported blood appeared to be utilized ultimately to make good the losses that ensued directly from the bodily withdrawal of blood from the circulation. These hypotheses appear to explain in the main the results of the second experiment also (see page 233). Nevertheless, it is quite probable that the influence of the anæsthetic was sufficient to make it impossible to interpret correctly the significance of the small period balances as well as the remaining results that otherwise might be attributed wholly to the effects of the hemorrhages. On the other hand, it is not very probable that the uniformities in the results of our two experiments (see page 239) are mere coincidences or due primarily to the action of the anæsthetic (see Table XI).

*Sulphur catabolism.* — For lack of time our quantitative determinations of sulphur in the excreta of both our experiments were confined to "period urines" and "period faeces." The period balances, as shown in Table VIII, were always small "plus balances." The daily average "plus balances" recorded in Table VIII make it apparent that the internal hemorrhages had no appreciable effect on the total sulphur catabolism per period. The records fail to show

close parallelism between the total nitrogen and sulphur outputs except in the first and last three periods (see Table XI).

The analytic data for urinary and fæcal sulphur of the first experiment are summarized in Table IX.

*Phosphorus catabolism.* — Our quantitative determinations of phosphorus in the excreta, as in the case of sulphur, were confined in both our experiments to "period urines" and "period fæces." The period balances, as shown in Table VIII, were plus balances in all the periods except the third and sixth. The average daily balances recorded in Table VIII indicate that the internal hemorrhages had no uniform effect on the phosphorus catabolism. The results of our analyses make it evident that total phosphorus catabolism per period was not perfectly concordant with the total catabolism of nitrogen and sulphur during the same periods. Facts in this relation are shown in Table XI.

Our analytic data for urinary and fæcal phosphorus of the first experiment are summarized in Table X.

#### IV. SECOND EXPERIMENT.

**Animal.** — The second dog was a short-haired mongrel male, not as lively as the first, but in good condition.

**Diet.** — The character and quantitative features of the dog's diet are indicated in Table XII. The diet was uniform throughout the entire experiment.

**Preparatory period.** — During a preparatory period of seven days, the dog was brought to approximate nitrogenous equilibrium, after which, on April 28 (1906), we started the period of special observations, as recorded in Table XIII. The dog then weighed 6.75 kilos. The records were continued daily for fifty-seven days. The experiment was divided into seven periods.

**First period. Normal conditions. Maintenance of approximate nitrogenous equilibrium. Days, 1-7; April 28-May 4, 1906.** — Weight was maintained at 6.75 kilos daily for seven days.

**Second period. First hemorrhage. Days, 8-17; May 5-14.** — The operation was conducted without difficulty. Blood was drawn in three portions, and at once poured into the peritoneal cavity, as in the first experiment.

**Schedule of operations.** — Anæsthesia started, 2.50 P. M. Leg opened, 3.10. Artery cannulized, 3.22. Abdomen opened, 3.25. Blood introduced at 3.30, 3.37, and 3.47. Abdomen closed, 4.06. Anæsthetic continued, 4.08. Leg closed, 4.15. Dog up, 4.30.

**Blood.**—The quantity of blood transferred was 262 grams, an amount equal to 3.88 per cent of the weight of the dog just before the operation.

**Third period. Second hemorrhage. Days, 18-29; May 15-26.**—The operative procedure was uneventful. Opposite sides were used for the exposures, — right leg and left side of abdomen.

TABLE XII.  
COMPOSITION OF THE DAILY DIET.<sup>1</sup>

	Hashed lean beef.	Cracker meal.	Lard.	Bone ash.	Water.
Daily amounts . . .	gm. 105.0000	gm. 31.0000	gm. 21.0000	gm. 7.0000	c.c. 280
Nitrogen . . . . .	3.8690	0.4805	0.0055	0.0021	
Sulphur . . . . .	0.2989	0.0406	0.0063	0.0042	
Phosphorus . . . . .	0.2360	0.0415	0.0180	1.2450	
TOTALS FOR EACH DAILY MIXTURE.					
Total weight.	Nitrogen.	Sulphur.	Phosphorus.		
gm. 444	gm. 4.3570	gm. 0.3500	gm. 1.5405		

<sup>1</sup> Several lots of meat were used. These were found to vary so slightly in composition that, for purposes of computation, an average was taken of the various analyses (HAWK and GIES: *Loc. cit.*). As the dogs were not kept in perfect nitrogenous equilibrium, this course did not materially affect our deductions.

**Schedule of operations.**—Anæsthesia started, 3.30 P. M. Artery cannulized, 3.50. Abdomen opened, 3.55. Blood introduced, 3.55, 4.00, 4.04, 4.09. Abdomen closed, 4.16. Anæsthetic discontinued, 4.16. Leg closed, 4.25. Dog up, 4.45.

**Blood.**—The quantity of blood transferred was 354 grams, an amount equal to 5.56 per cent of the weight of the dog just before the operation. This was the largest hemorrhage of the series and carried the dog to his cardiac and respiratory limits. The animal, as usual, recovered quickly in spite of the large quantity of blood transported.

**Fourth period. Third hemorrhage. Days, 30-36; May 27-June 2.**—Operation was conducted as usual, and was without incident except a little delay in closing the abdomen.

*Schedule of operations.* — Anæsthesia started, 4.25 P. M. Leg opened, 4.47. Artery cannulized, 5.02. Abdomen opened, 5.10. Blood drawn, 5.13, 5.16, 5.19. Abdomen closed, 5.45. Anæsthetic discontinued, 5.50. Leg closed, 6.00. Dog up, 6.20.

*Blood.* — The quantity of blood transferred was 232 grams, an amount equal to 3.5 per cent of the weight of the dog at the beginning of the period.

**Fifth period. Fourth hemorrhage. Days, 37-40; June 3-6.** — The dog was subjected to a heavy hemorrhage after but a short period for recovery from the previous transfer. Operation was without incident, but the animal required constant watching, for the cardiac action was weak. Greatly increased coagulability of the blood required withdrawal of small amounts at a time.

*Schedule of operations.* — Anæsthesia started, 2.15 P. M. Leg opened, 2.35. Cannula in artery, 3.02. Abdomen opened, 3.10. Blood drawn, 3.12, 3.18, and 3.25. Abdomen closed, 3.44. Anæsthetic discontinued, 3.50. Leg closed, 3.56. Dog up, 4.10.

*Blood.* — The quantity of blood transferred was 262 grams, an amount equal to 4.19 per cent of the dog's weight at the beginning of the period.

The dog showed the strain and was not very lively at any time during this short period.

**Sixth period. Fifth hemorrhage. Days, 41-47; June 7-13.** — This hemorrhage also followed a short period for recovery. The amount of blood transferred was also large, especially in view of the heavy drain upon the system that occurred four days previously. The usual operation was carried out, but it was difficult to find an artery sufficiently large in either hind leg without invading the pelvis.

*Schedule of operations.* — Anæsthesia started, 4 P. M. Leg opened, 4.18. Cannula in artery, 4.36. Abdomen opened, 4.45. Blood drawn, 4.48, 4.50, and 4.52. Abdomen closed, 5.13. Anæsthetic discontinued, 5.15. Leg closed, 5.24. Dog up, 5.35.

*Blood.* — The quantity of blood transferred was 288 grams, an amount equal to 4.66 per cent of the dog's weight at the commencement of the period.

The dog's vitality was greatly diminished by the hemorrhage.

**Seventh period. Sixth and seventh hemorrhages. Days, 48-57; June 14-23.**

— Two internal hemorrhages were effected on successive days, after a short period for recovery from the preceding (fifth) hemorrhage. This plan caused excessive removal of blood from the circulation and nearly resulted in the death of the animal after each transfer. It was apparent that we had carried internal hemorrhage by our method as far as it was possible to take it without

TABLE XIII.

SOME OF THE DAILY RECORDS OF THE SECOND METABOLISM EXPERIMENT.<sup>1</sup>

First Period. — Maintenance of approx. nitrogenous equilibrium. April 28–May 4, 1906.							
Day.	Body weight.	Urine.					Fæces dry.
No. in the experiment.		Volume.		Sp. Gr.	Nitrogen.		
		Daily.	Period av. to date.		Daily.	Period av. to date.	
1	kilos. 6.75	c.c. 285	c.c. ....	1.015	gm. 3.97	gm. ....	gm. 4
2	6.75	290	287	1.014	3.67	3.82	12
3	6.75	305	293	1.015	4.20	3.95	14
4	6.75	300	295	1.017	4.67	4.13	16
5	6.75	250	286	1.012	2.89	3.88	11
6	6.75	325	293	1.014	4.10	3.92	5
7	6.75	280	291	1.015	3.61	3.87	25
Second period. — Effects of operation and first hemorrhage (3.88 per cent). May 5–14, 1906.							
8	6.62	270	....	1.028	5.48	....	5
9	6.54	400	335	1.013	5.09	5.28	7
10	6.66	205	292	1.016	3.65	4.74	31
11	6.58	210	246	1.027	4.24	4.64	18
12	6.52	225	262	1.020	4.38	4.57	19
13	6.52	260	262	1.018	4.10	4.49	11
14	6.45	300	267	1.024	6.69	4.80	16
15	6.45	220	261	1.014	3.10	4.59	0
16	6.42	220	255	1.019	4.31	4.56	8
17	6.38	285	260	1.019	5.33	4.64	20
Third period. — Effects of operation and second hemorrhage (5.56 per cent). May 15–26, 1906.							
18	6.35	175	....	1.026	3.84	....	9
19	6.21	280	227	1.018	4.95	4.39	23
20	6.27	230	228	1.019	4.57	4.45	7
21	6.25	275	240	1.019	5.30	4.66	16
22	6.30	235	239	1.013	3.28	4.39	2
23	6.30	240	239	1.019	4.80	4.46	23
24	6.31	270	243	1.015	4.27	4.43	15
25	6.30	290	249	1.015	4.05	4.38	13
26	6.29	255	250	1.016	3.60	4.30	29
27	6.26	300	255	1.017	4.87	4.35	17
28	6.30	255	255	1.013	3.20	4.25	20
29	6.37	225	253	1.014	2.72	4.12	10
<sup>1</sup> Facts regarding the daily food are given in Table XII.							



TABLE XIII (continued).

Fourth period. — Effects of operation and third hemorrhage (3.5 per cent). May 27–June 2, 1906.							
Day.	Body weight.	Urine.					Fæces dry.
No. in the experiment.		Volume.		Sp. Gr.	Nitrogen.		
		Daily.	Period av. to date.		Daily.	Period av. to date.	
30	kllos. 6.28	g.c. 290	g.c. ....	1.017	gm. 4.43	gm. ....	gm. 16
31	6.23	320	305	1.016	4.97	4.70	14
32	6.22	310	307	1.016	4.42	4.61	8
33	6.22	300	305	1.012	3.97	4.45	2
34	6.22	265	297	1.015	3.92	4.34	17
35	6.26	300	298	1.016	5.25	4.49	10
36	6.24	295	297	1.014	4.37	4.48	14
Fifth period. — Effects of operation and fourth hemorrhage (4.19 per cent). June 3–6, 1906.							
37	6.13	195	....	1.024	4.23	....	14
38	6.12	280	238	1.010	3.56	3.89	15
39	6.20	185	220	1.012	2.27	3.35	9
40	6.17	295	239	1.018	5.56	3.91	16
Sixth period. — Effects of operation and fifth hemorrhage (4.66 per cent). June 7–13, 1906.							
41	6.14	255	....	1.023	5.07	....	17
42	6.14	250	250	1.019	4.47	4.77	16
43	6.12	315	273	1.014	4.46	4.67	3
44	6.12	230	262	1.017	4.45	4.61	11
45	6.10	310	272	1.015	4.76	4.64	16
46	6.07	270	271	1.018	4.62	4.64	17
47	6.09	210	263	1.013	2.71	4.36	21
Seventh Period. — Effects of operation and sixth and seventh hemorrhages (1.6 per cent and 1.8 per cent). June 14–23, 1906.							
48 <sup>1</sup>	5.95	310	....	1.018	4.56	....	20
49 <sup>2</sup>	5.90	260	285	1.031	6.19	5.37	0
50	5.83	195	255	1.023	3.95	4.90	22
51	5.77	290	264	1.014	3.83	4.63	30
52 }	5.77	480	256	1.016	7.45	4.33	46
53 }							
54 }	5.78	470	251	1.016	6.48	4.06	34
55 }							
56 }	5.85	550	256	1.014	6.25	3.87	46
57 }							
<sup>1</sup> Sixth hemorrhage, 1.6 per cent.				<sup>2</sup> Seventh hemorrhage, 1.8 per cent.			

killing the dog. It seemed probable also that metabolic effects of internal hemorrhage, if measurable ones ensued, would be at their maximal points. The operations were difficult because both legs had been worked over so much that operative fields were scarce; besides the blood coagulated with special readiness.

*Schedule of operations. Sixth hemorrhage, June 14.* — Anæsthesia started, 4.10. Leg opened, 4.45. Cannula in, 5.25. Abdomen opened, 5.32. Blood drawn, 5.36, 6.17, and 6.20. Anæsthetic discontinued, 6.30. Abdomen and leg closed, 6.37. Dog up, 6.40.

*Blood.* — The quantity of blood transferred was 100 grams, an amount equal to 1.6 per cent of the dog's weight at the beginning of the period.

*Seventh hemorrhage, June 15.* — Anæsthesia started, 4.15 P. M. Leg opened, 4.25. Cannula in artery, 5.30. Abdomen opened, 5.35. Blood in, 5.40–6.00. Abdomen closed, 6.20. Anæsthetic discontinued, 6.20. Leg closed, 6.30. Dog up, 6.35.

*Blood.* — The quantity of blood transferred was 108 gm., an amount equal to 1.8 per cent of the weight of the dog before the operation.

As was stated above, each of these hemorrhages nearly resulted fatally. The dog remained on a low plane of vitality for several days, but, with unimpaired appetite, gained steadily. He was kept under general observation until the following December.

**Autopsy.** — The dog was killed when he had regained his normal weight, nearly six months after the last operation.<sup>18</sup> The abdomen and viscera were in excellent condition; there was no free fluid, and only a slight brownish tint to the omentum. Heart and lungs, as in the first dog, were normal. The intestines were free from adhesions, but one delicate band passed from the stomach fundus to the abdominal wall, and a similar one from the liver to the right parietes. No such adhesions occurred in the dog of the first experiment.

**Supplementary data.** — The remarks on page 218 apply to this experiment also.

**Analytic results.** — Summaries of the results of the second experiment are given in Tables XIII–XXII.

**Discussion of results. Decline in body weight.** — The dog lost weight steadily after the first hemorrhage, but there were no abrupt falls and very few recoveries. The loss after fifty-seven days and seven hemorrhages was only 13 per cent of the original weight (see page 238).

\* The diet meanwhile had been practically the same as that fed daily during the experiment.

*Fluctuations in the volume of urine.* — As in the previous experiment, urine output was low at first, rising to the maximum usually on the second day. In the last period the highest output occurred on the first day, as was the case after the last hemorrhage of the first experiment, and probably for identical reasons.

TABLE XIV.  
TOTAL URINE VOLUMES ON THE EARLIER DAYS OF THE PERIODS.

Conditions.	Period no.	Volume of urine.			
		First day.	Second day.	Third day.	Fourth day.
Normal . . . . .	I	c.c. 285	c.c. 290	c.c. <b>305</b>	c.c. 300
First hemorrhage . . . . .	II	270	<b>400</b>	205	210
Second hemorrhage . . . . .	III	175	<b>280</b>	230	275
Third hemorrhage . . . . .	IV	290	<b>320</b>	310	300
Fourth hemorrhage . . . . .	V	195	<b>280</b>	185	295
Fifth hemorrhage . . . . .	VI	255	250	<b>315</b>	230
Sixth and seventh hemorrhage	VII	<b>310</b> <sup>1</sup>	<b>280</b> <sup>2</sup>	195	290
<sup>1</sup> After sixth hemorrhage.		<sup>2</sup> After seventh hemorrhage.			

*Specific gravity of the urine.* — This varied from 1.012 to 1.031. It was always immediately increased by the hemorrhages, and exhibited considerable variation, thereafter, during a period. Specific gravity was generally lowest at the ends of the hemorrhage periods.

*Nitrogen catabolism.* — Elimination of urinary nitrogen was increased during the first day or two of nearly all the hemorrhage periods. Individual figures for urinary nitrogen were lower, as a rule, in the last three hemorrhage periods than in the first three periods following hemorrhages. The average daily urinary content of nitrogen was always lower by the end of a hemorrhage period (except the fifth) than at its beginning. The highest final daily average for a period was recorded after the first hemorrhage; the lowest after the last hemorrhages. These final averages were not very divergent.

In all but one case (fifth period) the average daily amount of

nitrogen, in the urine of the three days opening a period after hemorrhage, was higher than the average for the three days closing the preceding period.

TABLE XV.  
ANALYTIC TOTALS AND AVERAGES FOR NITROGEN IN EACH PERIOD.

Period number . . . .	I	II	III	IV	V	VI	VII
Days in the period . .	7	10	12	7	4	7	10
Conditions of the period	{ Nor- mal.	First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.	Sixth and seventh hemor.
Food . . . . .	30.49	43.57	52.28	30.49	17.42	30.49	43.57
Excreta . . . . .	28.98	49.32	53.91	32.58	16.80	32.55	40.86
Total balance . . . .	+1.51	-5.75	-1.63	-2.09	+0.62	-2.06	+2.71
Average daily balance .	-0.21	-0.57	-0.13	-0.30	+0.15	-0.29	+0.27

TABLE XVI.  
NITROGEN IN THE URINE, PER PERIOD.

Period number . . . .	I	II	III	IV	V	VI	VII
Days in the period . .	7	10	12	7	4	7	10
Conditions of the period	{ Nor- mal.	First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.	Sixth and seventh hemor.
Daily average . . . .	3.87	4.64	4.12	4.48	3.90	4.36	3.87
Average, first three days	3.95	4.74	4.45	4.61	3.89 <sup>1</sup>	4.67	4.90
Average, last three days	3.53	4.25	3.60	4.51	3.91 <sup>2</sup>	4.03	3.16
<sup>1</sup> Average of the first two days (four-day period). <sup>2</sup> Average of the last two days (four-day period).							

The small output of nitrogen in the fæces increased to a maximum in the third period and fell to a minimum in the last period. There was a moderate increase in the second period, as was the case with urinary nitrogen. A further slightly increased output of fæcal nitrogen in the third period was inverse to the fall of nitrogen in the urine, while nitrogen in the urine rose and in the fæces fell in the fourth period. In the fifth period there was an excre-

tory decrease of nitrogen in both urine and faeces. Elimination of nitrogen in the faeces continued to decrease quantitatively, *per diem*, to the end of the experiment, and was at or below normal during the last two periods, whereas the urinary quantities rose above and fell below the normal figure.

TABLE XVII.  
NITROGEN IN THE FÆCES, PER PERIOD.

Period number . . . .	I	II	III	IV	V	VI	VII
Days in the period . .	7	10	12	7	4	7	10
Conditions of the period	{Nor- mal.	First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.	Sixth and seventh hemor.
Total . . . . .	gm. 1.84	gm. 2.92	gm. 4.39	gm. 2.20	gm. 1.18	gm. 1.99	gm. 2.14
Daily average . . . .	0.26	0.29	0.36	0.31	0.29	0.28	0.21

TABLE XVIII.  
QUANTITATIVE FÆCAL ELIMINATIONS, PER PERIOD.

Period number . . . .	I	II	III	IV	V	VI	VII
Days in the period . .	7	10	12	7	4	7	10
Conditions of the period	{Nor- mal.	First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.	Sixth and seventh hemor.
Dry weight : period total	gm. 87	gm. 135	gm. 184	gm. 81	gm. 54	gm. 101	gm. 198
" " daily average	12	14	15	12	13	14	20

On comparing the total amounts of nitrogen per period in the food with those in the corresponding excreta (Table XV), it will be seen that the earlier hemorrhages were followed by small minus balances, the later hemorrhages (except the fifth) by small plus balances. This general result is in practical accord with the analogous outcome of the first experiment (see discussion, page 225).

*Sulphur catabolism.* — The changes in the excreted amounts of sulphur in urines for whole periods followed those of nitrogen in the same urines, though with relatively less variation, up to the last period, when the amount of sulphur rose as that for nitrogen fell. The decreased elimination of sulphur in the urine per day after the first hemorrhage was practically continuous to the fifth

period, after which there was a correspondingly cumulative increase to the end.

The figures for total sulphur catabolism show an extreme initial decrease. Later the period balances are very small, without showing regular effects of the hemorrhages. With two exceptions the period balances, as shown in Table XIX, were small "plus balances." The effects, if any, of the internal hemorrhages are not evident,

TABLE XIX.

SUMMARY OF ANALYTIC DATA FOR TOTAL SULPHUR AND PHOSPHORUS METABOLISM.

Period conditions.	SULPHUR.				PHOSPHORUS.			
	Total.		Balance.		Total.		Balance.	
	In-gested.	Ex-creted.	Total.	Daily average.	In-gested.	Ex-creted.	Total.	Daily average.
I. Normal . . .	gm. 2.45	gm. 2.18	gm. +0.27	gm. +0.0386	gm. 10.78	gm. 10.53	gm. +0.25	gm. +0.0357
II. First hemor. .	3.50	3.60	-0.10	-0.0100	15.40	16.16	-0.76	-0.0760
III. Second hemor.	4.20	4.01	+0.19	+0.0158	18.48	23.50	-5.02	-0.4183
IV. Third hemor.	2.45	2.32	+0.13	+0.0186	10.78	9.92	+0.86	+0.1230
V. Fourth hemor.	1.40	1.28	+0.12	+0.0300	6.16	6.56	-0.40	-0.1000
VI. Fifth hemor. .	2.45	3.14	-0.69	-0.0985	10.78	12.45	-1.67	-0.2386
VII. Sixth and seventh hemor.	3.50	3.07	+0.43	+0.0430	15.40	25.41	-10.01 <sup>1</sup>	-1.0010

<sup>1</sup> This excessive minus balance was due largely to the greater elimination of fæces in this period. See Table XVIII.

unless the output of sulphur was increased in variable degrees though not in sufficient amounts in more than two periods to change plus balances to minus balances. Total sulphur excretion for periods ran parallel with total nitrogen elimination for the same periods in all except the third and fourth (see Table XXII).

The analytic data for urinary and fæcal sulphur of the second experiment are summarized in Table XX.

*Phosphorus catabolism.*—The general trend of the output of phosphorus in the period urines was downward, there having been a sharp decrease in the second period, a partial recovery in the third and fourth periods, and then a decrease to a subnormal output in the last two periods.

TABLE XX.  
QUANTITATIVE SULPHUR EXCRETION, PER PERIOD.

TOTALS.							
Period number . . .	I 7	II 10	III 12	IV 7	V 4	VI 7	VII 10
Days in the period . .							
Conditions of the period	{Nor- mal.	First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.	{Sixth and seventh hemor.
Urine . . . . .	gm. 1.82	gm. 3.03	gm. 3.211	gm. 1.98	gm. 1.00	gm. 1.99	gm. 2.14
Fæces . . . . .	0.357	0.567	0.794	0.343	0.277	0.434	1.00
DAILY AVERAGES.							
Urine . . . . .	0.260	0.300	0.290	0.280	0.250	0.270	0.290
Fæces . . . . .	0.051	0.056	0.066	0.049	0.069	0.062	0.100

TABLE XXI.  
QUANTITATIVE PHOSPHORUS EXCRETION, PER PERIOD.

Period number . . .	I 7	II 10	III 12	IV 7	V 4	VI 7	VII 10
Days in the period . .							
Conditions of the period	{Nor- mal.	First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.	{Sixth and seventh hemor.
Urine . . . . .	gm. 2.06	gm. 1.79	gm. 2.45	gm. 1.86	gm. 0.83	gm. 1.39	gm. 1.90
Fæces . . . . .	8.47	14.36	21.05	8.05	5.73	11.05	23.51
DAILY AVERAGES.							
Urine . . . . .	0.29	0.17	0.20	0.26	0.20	0.19	0.19
Fæces . . . . .	1.21	1.43	1.75	1.15	1.43	1.58	2.35

The daily average elimination of phosphorus in the fæces decreased, as a rule, as the daily average output of phosphorus in the urine increased. The minimal excretion in the fæces of the fourth period corresponded with the maximum amount of excreted phosphorus in the urine after hemorrhage, whereas the maximum amount

of phosphorus in the fæces of the last period corresponded with the subnormal excretion of phosphorus in the urine.

The amounts of ingested phosphorus were less than those of excreted phosphorus in all but the first and fourth periods, *i. e.*, the balances, except in these two periods, were minus balances. This result is practically the opposite of that obtained in the first experiment.

TABLE XXII.  
SUMMARY OF PERIOD, AVERAGE DAILY BALANCES FOR NITROGEN, SULPHUR,  
AND PHOSPHORUS.

Period number . . .	I	II	III	IV	V	VI	VII
Conditions of the period	Normal.	} First hemor.	Second hemor.	Third hemor.	Fourth hemor.	Fifth hemor.	} Sixth and seventh hemor.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Nitrogen . . . . .	+0.210	-0.570	-0.130	-1.010	+0.150	-0.290	+0.270
Sulphur . . . . .	+0.182	-0.010	+0.016	+0.019	+0.030	-0.099	+0.043
Phosphorus . . . . .	+0.036	-0.076	-0.418	+0.123	-0.100	-0.239	-1.001

The figures for total phosphorus catabolism indicate that phosphorus elimination ran somewhat independently of both nitrogen and sulphur excretion, except in the first, second, and sixth periods. Facts in this relation are shown in Table XXII.

Our analytic data for urinary and fæcal phosphorus of the second experiment are summarized in Table XXI.

The general influence of the hemorrhages on phosphorus metabolism seems to have been in the direction of increased excretion, although the power of the anæsthetic to produce such effects must not be overlooked in an interpretation of these results.

#### V. DISCUSSION OF THE GENERAL RESULTS OF THE TWO EXPERIMENTS.

**Decline in body weight.** — The first dog lost 11 per cent after sixty-one days and five internal hemorrhages; the second dog lost 13 per cent after fifty-seven days and seven internal hemorrhages,



in comparison with a loss of 25 per cent after eighty-five days and five external hemorrhages, and 16 per cent after thirty-three days and four external hemorrhages, in the experiments by Hawk and Gies under similar feeding conditions. These facts certainly indicate that internal hemorrhage causes less drain upon the system than external hemorrhage.

**Fluctuations in the volume of urine.** — Tables III and XIV exhibit practically identical results, and show that the maximal volumes of urine were usually excreted on the second day after hemorrhage, in spite of the tendency of ether to cause greatly increased flow of urine on the day of anæsthesia, when anæsthesia is as light as that induced in these experiments. The outcome was evidently a resultant of the contrary immediate effects of ether anæsthesia and loss of blood from the vessels.<sup>19</sup>

**Changes in the specific gravity of the urine.** — Specific gravity was increased on the day of hemorrhage in each experiment, in nearly every instance, and fell with considerable fluctuation to low points at the ends of hemorrhage periods. After external hemorrhage, under similar conditions of anæsthesia, specific gravity either fell at first or was unchanged, but rose in a day or two to maximal points and declined to normal values at the ends of the periods.

**Effects on the fæces.** — Neither the weights (fresh or dry) of the average daily fæcal eliminations nor the corresponding fæcal contents of nitrogen, sulphur, and phosphorus varied sufficiently to show any marked influences of the hemorrhages. There was evidently no particular effect on digestion or on assimilation. There were apparently also no noticeable effects on the secretion into the alimentary tract of nitrogenous matter, such as mucus. These results accord with those of the related study of external hemorrhage.

**Nitrogen catabolism.** — In each of the two experiments slightly less nitrogen was eliminated than ingested during the normal period. The earlier hemorrhages were always followed by small minus period balances of nitrogen, the later hemorrhages (except in one instance) by small plus period balances of nitrogen (see Table XXIII). In a general way the total excretion of nitrogen throughout the entirety of each experiment was only slightly greater than the total amount ingested. Either ether anæsthesia or internal hemorrhage, or both, were devoid of pronounced disturbing effects

<sup>19</sup> See HAWK and GIES: *Loc. cit.*, p. 194, Table III, for facts in this connection pertaining to the effects of anæsthesia and of external hemorrhage.

on the general metabolism of the dogs in these experiments, as measured by us, or their influences were mutually antagonistic in the main and therefore not discernible for more than a day or two, if at all.

TABLE XXIII.

ANALYTIC TOTALS OF NITROGEN FOR ALL THE PERIODS OF EACH EXPERIMENT.

Period No.	FIRST EXPERIMENT.		SECOND EXPERIMENT.	
	Nitrogen in food.	Nitrogen in excreta.	Nitrogen in food.	Nitrogen in excreta.
I . . . . .	gm. 26.10	gm. 25.53	gm. 30.49	gm. 28.98
II . . . . .	83.52	89.43	43.57	49.32
III . . . . .	62.64	64.94	52.28	53.91
IV . . . . .	46.98	43.06	30.49	32.58
V . . . . .	52.20	50.45	17.42	16.80
VI . . . . .	46.98	46.50	30.49	32.55
VII . . . . .	....	....	43.57	40.86
Total . . . . .	318.42	319.91	248.31	255.00
Total balance . . .	- 1.49	....	- 6.69	....
Average daily balance	- 0.024	....	- 0.117	....

Elimination of urinary nitrogen was greater during the first day or two of practically all the hemorrhage periods. Individual figures for urinary nitrogen were lower, as a rule, in the last three hemorrhage periods than in the first three periods after hemorrhages. The average daily urinary elimination of nitrogen was usually lower by the end of a hemorrhage period than at its beginning. The highest daily average content of nitrogen in the urine of a period was recorded in each experiment at the end of the first hemorrhage period. In nearly all the hemorrhage periods the average daily amount of nitrogen, in the urine of the three days that opened a period after hemorrhage, was higher than the average for the three days that closed the preceding period.

In the first experiment the average daily elimination of nitrogen in the urine was greater after all but two of the hemorrhages than during the normal period. In the same experiment the nitrogen

of the fæces exceeded the normal fæcal elimination after only two of the five hemorrhages. In the second experiment the average daily excretion of urinary nitrogen after *each* hemorrhage was equal to or greater than that of the normal period. The same was true of the fæces, except for the last period.

During the early stages of such experiments as these, when the ordinary blood surplus is not too greatly reduced, it is probable that appreciable proportions of the absorbed nitrogenous and other matters from the transported blood are not utilized but quickly excreted, as when an excess of protein food is taken. Later, in experiments of this kind, after thorough depletion of the circulating blood surplus, the absorbed matters from the transposed blood, whether changed or not, are probably more completely utilized and retained after their return to the circulation.

**Sulphur catabolism.** — In the first experiment there was, in each period, a retention of sulphur — all the period average daily balances were small “plus balances” in fairly close agreement. In the second experiment the balances were “plus balances” except after two of the seven hemorrhages. Retention of sulphur in the second experiment was greatest in the normal period.

In the first experiment the period average daily sulphur difference was smaller than that of the normal period after three hemorrhages, and larger than the normal balance after two hemorrhages. In the second experiment all the sulphur “plus balances” were much less after the hemorrhages than after the first period of normal conditions. In a very general way the total excretion of sulphur, as in the case of nitrogen, was slightly increased after internal hemorrhage. This result is in accord with the effect of external hemorrhage on sulphur catabolism. In both normal periods and in seven of the eleven hemorrhage periods the signs of the sulphur and nitrogen balances were the same (see Tables XI and XXII).

The urinary and fæcal eliminations of sulphur were, in terms of daily averages, only slightly affected by the hemorrhages in most instances.

**Phosphorus catabolism.** — In the first experiment there was retention of phosphorus in the normal period and in three of the five hemorrhage periods. In each of these three hemorrhage periods the amount of retained phosphorus was greater than that in the normal period. In the second experiment there was again retention of

phosphorus in the normal period, but in only one of the six hemorrhage periods. The phosphorus results for the two experiments were therefore not in accord. In a very general way, however, phosphorus excretion was somewhat increased after hemorrhage, in harmony with the results for nitrogen and sulphur. In their study of the effects of external hemorrhage Hawk and Gies found that there was "a variable effect on the elimination of phosphorized substances, though mainly a decreased excretion of the latter."

The daily average urinary and faecal eliminations of phosphorus were not as uniform as those of sulphur after the hemorrhages. The large quantity of phosphate in the bone ash of the diet, with the increased difficulties of accurate analysis, may have had some influence in this connection.

In both normal periods and in six of the eleven hemorrhage periods the signs of the phosphorus and nitrogen balances were the same. In both normal periods and in six of the eleven hemorrhage periods the signs of the phosphorus and sulphur balances were identical. In both normal periods and in four of the eleven hemorrhage periods the signs of the nitrogen, sulphur, and phosphorus balances were in agreement (see Tables XI and XXII).

**General observations.** — The mammalian organism probably has a fairly large reserve supply of blood. *External* loss of a moderate amount of this surplus from a healthy animal seems to have little or no harmful effect. Hurtful influences of hemorrhage are less apt to be exerted, all other conditions being equal, if the blood passes from the vascular system to the peritoneal cavity, instead of away from the body. It is only when the vascular drain is comparatively great, in cases of internal hemorrhage, or under ordinary conditions of excessive external hemorrhage, that decisive modification of general metabolic factors is effected. Hemorrhage in any degree naturally results in metabolic disturbances, slight though they may be.

Neither sugar nor protein, in abnormal quantities, could be detected in any of the urines of these experiments.

We could not carry our experiments far enough to obtain answers to the open questions connected with the ultimate causes of some of the more important excretory phenomena we have observed, but it is Dr. Gies's intention to continue his study of this subject with more intimate inquiries into these and related matters. For this reason we refrain from indulging the temptation to offer additional hypotheses in explanation of the metabolic data herein recorded.

## VI. PRACTICAL APPLICATION.

It would seem that with an organism in healthy condition a hemorrhage which did not endanger life, *e. g.*, 1 per cent to 2 per cent of body weight, would do little or no harm, especially if intraperitoneal. Repeated losses of blood would cause increasing damage, but relatively less if intra-abdominal instead of external. It is only after great cumulative losses or after an overwhelming single hemorrhage that the system does not recover promptly, if at all. In the case, then, of such losses of blood as occur from a ruptured ectopic pregnancy or ruptured spleen or liver, with the hemorrhage stopping without surgical interference, the blood, *per se*, may be not only devoid of harm, but of special use in the body. Such use would be influenced, however, by possible infections and adhesions. If surgical intervention were contemplated in such cases, the surgeon would have to consider these possibilities and the probable effects. With the patient placed so that the blood would gravitate toward the diaphragm, *i. e.*, with feet raised, the blood would collect where absorption would be very rapid, where infection could best be met, and where, if adhesions should form, they would do a minimum of harm, *i. e.*, between the suphrenic organs and away from the intestines and pelvic viscera. The more rapid the absorption, the less the likelihood of adhesions. We fully appreciate the fact, however, that observations of this kind pertaining primarily to the dog cannot be applied automatically to man. Very thorough and very discriminating study of human cases must be conducted before such conclusions can be accepted for practical guidance.

## VII. SUMMARY OF GENERAL CONCLUSIONS.

After internal hemorrhage equal to from 2.85 to 5.56 per cent of the body weight of dogs, the weight of the animals declined perceptibly. The maximal volume of urine was usually excreted on the second day after internal hemorrhage. The specific gravity of the urine was increased on the days of internal hemorrhage. There were no constant effects, if any, on the reaction of the urine.

There were no marked influences of internal hemorrhage on the weight or consistency of the fæces nor on the fæcal eliminations of nitrogen, sulphur, and phosphorus. No particular influence was

exercised on digestion or assimilation. There was no appreciable effect on the secretion into the alimentary tract of nitrogenous matter, such as mucus.

Either ether anæsthesia or internal hemorrhage, or both, were devoid of pronounced disturbing influences as measured by us, on the general metabolism of the dogs in these experiments, or their effects were mutually antagonistic in the main and, therefore, not discernible for more than a day or two, if at all. In a very general way the total excretions of nitrogen, sulphur, and phosphorus were slightly increased after the internal hemorrhages.

Neither sugar nor protein, in abnormal quantities, could be detected in any of the urines of these experiments.

Comparative observations are summarized on p. 238.

We gladly embrace this opportunity to thank Professor William J. Gies for constant supervision of the work, which he suggested and guided. We also wish to acknowledge our indebtedness to Drs. Welker and Berg and Mr. Seifert for much helpful assistance.

# PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES OF THE URETER. III.<sup>1</sup>

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## I. ON TRANSMISSION OF PRESSURE FROM THE BLADDER TO THE KIDNEY.

**Introductory.**—It is often said that “distention of the bladder seems to cause congestion of the kidneys and, when frequent and long continued, may even be etiological of nephritis.” At various times clinicians have asked whether I have noticed regurgitation of urine into the ureter from the bladder.

Although I have been unable to find any exception to the statement that the so-called uretero-vesicular valve is normally quite competent, such questions as the one mentioned above indicate the existence of some doubt as to whether pressure in the bladder may have an effect on the kidneys by direct transmission through the ureter, or only by indirect nervous influence. Publication of some of my notes regarding this matter may therefore be of interest.

**Experimental.**—My first observation in this connection was made during an investigation of normal ureteral pressure and its relation to the peristaltic movements of the ureter in the dog.<sup>2</sup>

<sup>1</sup> The first paper was published in this journal, 1906, xvii, p. 392; the second paper appeared in the New York medical journal, 1907 (August 10).

<sup>2</sup> LUCAS: Proceedings of the Society for Experimental Biology and Medicine, 1905, ii, p. 61; also Science, 1905, xxi, p. 721; American medicine, 1905, ix, p. 744; Medical news, 1905, lxxxvii, p. 87.

In that series of experiments a cannula, maintained without ligatures and not materially interfering with either the flow of urine or the peristalsis of the ureter, was inserted in the ureter at various locations between the kidney and the bladder. It was connected with a water manometer, fitted with float and style to record, on a revolving smoked drum, the intra-ureteral pressure and the effect of the peristaltic waves on that pressure. It was noted that if a kink in, or compression of the ureter below the cannula prevented flow from the ureter, a proportionate increase was registered in the amount of intra-ureteral pressure and the number of peristaltic contractions, — results that confirmed the related conclusions of Sokoloff and Luchsinger.<sup>3</sup>

An unsuccessful attempt was made to cause a more rapid rise in intra-ureteral pressure than was obtainable by the collection above the clamp of urine secreted by the kidney, by squeezing the well-filled bladder with the hand. This was done in a number of animals and frequently repeated in the same animal. In all but one case it was found to be *impossible* to cause in this way increased intra-ureteral pressure. In the one exceptional case the left uretero-vesicular valve seemed to be deficient. The right valve, however, was entirely competent. The force exerted on the bladder in these experiments was sufficient in each case to overcome the compressor-urethra muscle and empty the bladder, or, when the urethra was clamped, to burst the bladder. In the ureter *in situ*, the animal being narcotized with morphine, the rate of ureteral peristalsis recorded on a smoked drum was, as a rule, increased by the manipulation. This increased contraction was apparently caused by nervous influence and not by mechanical distention (Protocol No. 1).

The competency of the uretero-vesicular valve was noted in five different experiments in which, also, the ureteral pressure was observed as described in Protocol No. 1. The nozzle of a ten-ounce metallic syringe was firmly ligated into the bladder or urethra, and salt solution injected until the bladder burst, without affecting the ureter pressure in any instance (Protocol No. 2). In five perfusion experiments the recording apparatus was not attached, the ureter being merely inspected and palpated. Pressure exerted as described in Protocol No. 2 did not cause dilatation of the ureter. In all

<sup>3</sup> SOKOLOFF and LUCHSINGER: *Archiv für die gesammte Physiologie*, 1881, xxvi, p. 464.



these experiments the freedom of flow of the perfusion fluid through the kidney vessels was never retarded in the slightest degree by pressure exerted in the bladder (Protocol No. 3). When pressures of 20 to 40 cm. of water were produced in the ureter by injection of Ringer solution through a cannula inserted above the ureterovesicular valve, the venous flow from the kidney was markedly diminished and the distention of the ureter could be plainly seen and palpated (see Fig. 1).

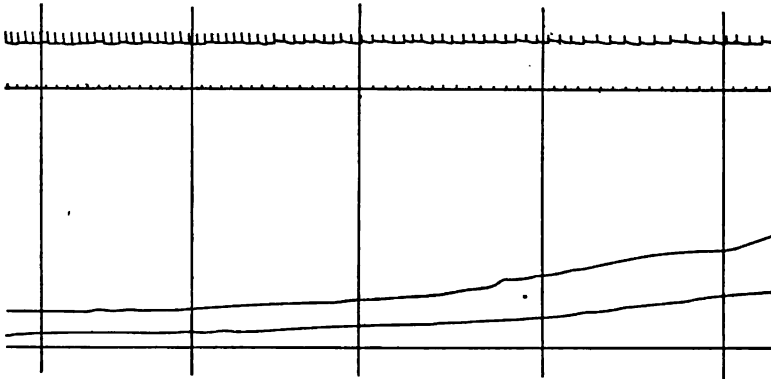


FIGURE 1. Upper tracing, drops of fluid from cannula in renal vein. Second tracing from top, time marked in seconds. Third tracing from top, from *L* cannula in middle portion of the ureter. Fourth tracing from top, pressure in renal pelvis registered through a trocar cannula. Straight line, base line for both pressure curves.

A series of experiments were performed by Dr. Burton-Opitz and myself, in the Physiological Laboratory of the College of Physicians and Surgeons, on the circulation of the blood in the kidneys of dogs anesthetized with ether. We investigated the effect of various pressures exerted at different places in the ureter, and in the bladder, on the rate of blood flow through the kidney, as measured with the Burton-Opitz stromuhr. It was found that the amount of pressure necessary to almost absolutely suppress the flow through the kidney when exerted in the ureter varied directly with the nearness of insertion of the cannula to the pelvis of the kidney. That variation of pressure ranged between 10 to 50 mm. of mercury.<sup>4</sup>

We were unable to note any change in the rate of flow of blood through the kidney in the experiments in which we recorded the

<sup>4</sup> BURTON-OPITZ and LUCAS: Proceedings of the Society for Experimental Biology and Medicine, 1908, v, p. 44.

flow from one kidney while special pressure was exerted in the opposite ureter. The results obtained by pressure in the bladder, which was accomplished by compressed air injected at different constant pressures through the urethra, and tried in increasing degrees up to one sufficient to burst the bladder, were also negative.

In these experiments the animals had previously been subjected to various other tests: to prolonged anesthesia with ether and chloroform; their abdominal contents had been exposed and manipulated; and often the renal nerves isolated and stimulated by electricity. Consequently a reflex nerve connection between bladder and kidney or between the two kidneys might easily have been interfered with or destroyed.

In all experiments in which the flow of blood through a kidney was reduced by pressure in its ureter, the blood flow rapidly returned to normal when the pressure was released.

When the bladder was removed from an animal and water injected into it through the urethra until it burst, no leakage was, as a rule, produced through the ureters.

**Conclusions.** — The results of these precise experiments accord with the experience of many a boy who has observed that a pig's bladder can be inflated with air by means of a quill inserted in the urethral opening; that such inflation can be made permanent by ligation of the urethra, no attention to the ureters being required; and that the bladder thus distended can be used as a football for days. This shows without doubt that the normal uretero-vesicular valves are entirely competent, and that they wholly prevent the slightest reflux of urine under any degree of pressure which can obtain in the bladder.

Therefore, as has been suggested above, if a continuous or frequently distended bladder has a deleterious effect on the kidney, this effect must be brought about, not by any direct transmission of pressure from the bladder to the kidney, but entirely by a nervous mechanism.

The latter will be considered more fully in a later research.

**Protocols. I.** Dog; weight, 24.56 kilos. Milk diet for twenty-four hours before the beginning of the experiment. Morphine (6 mg. per kilo) was injected hypodermically at 9.45 A. M., June 23, 1906. 11 A. M. Animal profoundly narcotized. A trocar cannula was introduced through the cortex and medulla of the kidney so

that it just entered the renal pelvis. The cannula was retained in place by a purse-string suture around the point of puncture of the capsule of the kidney. The urine aspirated fresh from the bladder was injected through the cannula, and the patency of the cannula and the ureter ascertained. An improved T cannula was inserted in the lower third of the ureter. Each cannula was connected with a water manometer and the pressure changes were recorded by means of an Emerson float,<sup>5</sup> on the smoked drum of a kymograph. At first a slightly positive pressure was recorded from the cannula in the straight portion of the ureter, and also from the cannula in the pelvis of the kidney. After a saline infusion of about 400 c.c. in the femoral vein, the amount of secreted urine and the rate of peristalsis were increased, but the intra-ureteral pressure was decreased. The bladder, which was distended with urine, was grasped by the hand, and pressure gradually exerted until the sphincter was overcome and the urine released. This had no effect on the pressure recorded by either manometer, but the irritation of the bladder called forth an increased peristalsis. After the urine had escaped, the pressure recorded by the cannulas remained about the same as previously. However, the variations in pressure caused by each peristaltic contraction were not so great for a time as they had been, but subsequently the waves resumed the original size.

2. Bull dog; weight, 16.8 kilos. Morphine, 0.168 gram at 9.40 A. M., July 3, 1905. Several additional small doses of morphine between 10.40 and 11.20. Two cannulas in the ureter of the right kidney: one, a trocar cannula through the cortex and medulla of the kidney into the renal pelvis, the other an improved T cannula, were inserted at the junction of the upper and middle thirds of the ureter. A straight glass cannula was inserted in the left ureter for collecting and measuring the flow of urine.

As the animal seemed to exhibit special tolerance for morphine, a small amount of chloroform was administered from time to time. A positive pressure of 2 cm. of water was registered from the straight portion at times when the chloroform was used, which caused retardation of the muscular action of the ureter.<sup>6</sup> As the effect of the chloroform wore off, the pressure in the pelvis of the kidney increased, and the pressure in the straight portion of the ureter fluctuated about a neutral point. One gram of diuretin in 30 c.c. of physiological salt solution was infused in the femoral

<sup>5</sup> EMERSON: Proceedings of the Society for Experimental Biology and Medicine, 1904-1905, ii, p. 38.

<sup>6</sup> LUCAS: New York medical journal, August 10, 1907.

vein. It gave rise to a flow from the left kidney of 1 c.c. of urine in eight minutes. The tip of a ten-ounce hand syringe was inserted at the urethro-vesicular opening and securely ligated in place. The urethra was clamped, and salt solution was injected into the bladder until the bladder burst. No increase in the pressure in the ureter was shown by the manometer.

3. February 18, 1908. — The kidney, ureter, and bladder of a dog were collectively removed. The cannula and recording instruments were adjusted as described in Protocol No. 2. Ringer solution was perfused into the renal artery at a constant pressure of 100 cm. The fluid from the renal vein was collected in a graduated cylinder, and record was made of the time which elapsed while 200 c.c. were collected, with the following results:

Time.	Fluid from the vein.	Tests applied.
	c.c.	
12.57 P.M.	0.0	....
1.11 P.M. (14 min.)	200.0	....
1.29 P.M. (18 min.)	200.0	The urethral outlet was clamped and the bladder was severely squeezed with the hand.
1.48 P.M. (19 min.)	200.0	....
2.09 P.M. (20 min.)	200.0	Ringer solution was injected into the bladder until it burst.
2.29 P.M. (20 min.)	200.0	....

NOTE. — The tendency of the vein flow gradually to decrease, as shown by the above figures, cannot be attributed in any degree to the manipulation of or pressure exerted in the bladder. It is a phenomenon observed during the first few hours of all kidney perfusion experiments, and has been accurately described and charted by Sollmann.<sup>7</sup>

## II. URETERAL PRESSURE.

**Introductory.** — The so-called ureteral pressure, which has been the subject of many studies, is, as pointed out by Henderson,<sup>8</sup> a misnomer. In the investigations of the so-called ureteral pressure it was not the pressure exerted by the ureter that was studied, but the pressure of the kidney secretion as observed by a manometer tied in the ureter. Sokoloff and Luchsinger, Henderson, and others observed that the ureter is capable of contractions sufficiently strong to overcome a very considerable intra-ureteral pressure. They stated that within physiological limits the rate of contraction was directly

<sup>7</sup> SOLLMANN: This journal, 1905, xiii, p. 249.

<sup>8</sup> HENDERSON: Journal of physiology, 1905-1906, xxxiii, p. 175.

proportional to the pressure. I have seen contractions in an isolated piece of the middle portion of a ureter from a small dog lift a pressure column of Ringer solution 92 cm. high. I have also recorded graphically contractions under a pressure of 86 cm. of the same solution, which recurred as often as four to five times per minute and, without decreased frequency, for forty minutes, at the end of which time the pressure was diminished. Pharmacological experiments were satisfactorily conducted on this ureter for some time thereafter.

As I pointed out in a previous paper,<sup>9</sup> the ureteral peristalsis is composed not only of wave motions, due to the shortening of both longitudinal and circular fibres, that travel from kidney to bladder, as described by Engelmann, but also of wave motions in at least that portion of the ureter contained in the renal pelvis, which are distinct and different from the contractions of the straight portion. I believe that further research will justify the general division of the ureter into the following two portions which are distinctly unlike each other in the character of their contractions and functions.

1. The funnel-shaped portion above the isthmus contained in the renal pelvis and probably partaking of the nerve distribution to the kidney.

2. The straight portion extending from the isthmus to the bladder, which may be subdivided into (a) an upper third, containing nerve endings in its wall; (b) a middle third, deficient in nerve endings; and (c) a lower third, adjacent to the bladder and partaking to some extent of the nerve distribution to the bladder.

I have often found that the ureter is capable of forcing urine into the bladder, even when sufficient pressure is gradually exerted in the bladder to burst it, no rise of pressure taking place in the ureter either from regurgitation or accumulation of urine secreted by the kidney.

Various investigations of the so-called ureteral pressure have shown that pressures varying from 5 to 20 cm. of water cause variable effects on the amount and constituents of the urine. Thus Steyrer<sup>10</sup> found that pathological closure of one ureter caused an increased flow, diminished specific gravity, and lowered freezing-point of the urine. Pfaundler<sup>11</sup> observed an increased flow in three

<sup>9</sup> LUCAS : This journal, 1906, xvii, p. 392.

<sup>10</sup> STEYRER : Beiträge zur chemischen Physiologie und Pathologie, 1902, ii, p. 312.

<sup>11</sup> PFAUNDLER : *Ibid.*, 1902, ii, p. 336.

dogs and in one woman under similar circumstances. Schwarz<sup>12</sup> noticed that pressures of 10–25 cm. of oil increased the flow of urine, but that greater pressure decreased the flow. Cushny<sup>13</sup> found, without exception, that a pressure of 19.5 cm. of water diminished urinary flow in rabbits. Sollmann<sup>14</sup> concluded from his experiments that the cause of the increase is due to forces vital and not mechanical.

The above-mentioned observations, in the light of my own experience with the ureter, lead me to raise this question: May not the living ureter antagonize transmission of pressure towards the kidney? I believe that definite conclusions on the effect upon the kidney of pressure exerted in the ureter, *in situ* or *überlebend*, are unwarranted before we know definitely how internal pressure influences the ureter. We should know not only the effect in the portion below the isthmus, but also in the portion in the renal pelvis, and the relation of the pressure in these two portions to each other, both normally and when artificially produced.

Before proceeding to a description and discussion of my experiments intended to answer this question, I wish to emphasize one point. In the above statement, *überlebend* (i. e., surviving) is used advisedly. The maintenance for many hours of vital contractile activity in the ureter when excised has made it a very satisfactory subject for the study of many points regarding involuntary muscle tissue. Not long ago I published a tracing showing the effect of caffeine on an excised ureter which had been kept in physiological salt solution for five hours after the animal had been killed by pithing.<sup>15</sup> Subsequently, in studies on the ureter and kidney which had been excised jointly and together placed in warm Ringer solution, contraction waves occurred with surprising rapidity and strength during perfusion of the kidneys with Ringer solution twenty-seven hours after their removal from the animal. These waves were graphically recorded. This observation, it will be noted, was made on the second day of the experiment. The temperature of the bath had been allowed to fall to that of the room before the end of the first day and the perfusion fluid had been withheld nineteen hours. Moreover, the peristalsis had been en-

<sup>12</sup> SCHWARZ: *Centralblatt für Physiologie*, 1902, xvi, p. 281.

<sup>13</sup> CUSHNY: *Journal of physiology*, 1902, xxviii, p. 431.

<sup>14</sup> SOLLMANN: *This journal*, 1905, xiii, p. 276.

<sup>15</sup> LUCAS: *New York medical journal*, August 10, 1907.

tirely inhibited at the end of the first day's experiment by the use of barium chloride, my intention being to study on the second day the effect of pressure on the dead ureter and kidney as compared with that on the living ureter and kidney on the previous day. Such observations emphasize the vigorous and prolonged vital activity of the excised ureter, — a fact in harmony with similar qualities of the excised kidney, as has been pointed out by Sollmann.<sup>16</sup>

**Experimental. Methods.** — The present experiments were made on dogs only. As a rule large animals were selected. The ureter was connected with a recording apparatus by

(A) An improved cannula made of two portions, introduced into the straight segment of the ureter. That part of the cannula between which the vessel wall was clamped was made of half cylinders, the perpendicular cylindrical portions being set nearer one end of each than the other.

(B) A trocar with a blunt obturator was introduced into the renal pelvis and pushed through the cortex and medulla of the kidney so that it just entered the renal pelvis.

In the paper on the peristalsis of the ureter two tracings were reproduced for the purpose of illustrating the regularity and persistence of ureteral peristalsis. At that time no significance was attached to the fact that, although the tracings were registered by a water manometer, the curve recorded a minimum of 3 mm. and a maximum of 5 mm. positive pressure in the straight portion of the ureter. Throughout the entire period of the three hours that intervened between the two tracings, a constant pressure of 11 cm. of urine was maintained at the distal end of the ureter<sup>17</sup> connected with a vertical glass tube from the upper end of which the urine escaped. In that particular experiment the trocar was not introduced into the renal pelvis. The observation suggests, however, that there was no transmission of pressure to the kidney through the ureter.

In fourteen experiments in which the trocar was placed in the renal pelvis an L cannula was inserted at the same time in the straight portion of the ureter at various locations between the isthmus and the bladder, and pressure was exerted in the outlet cannula (inserted just above the bladder) by one of the following three methods:

<sup>16</sup> SOLLMANN: This journal, 1905, xiii, p. 243.

<sup>17</sup> LUCAS: This journal, 1906, xvii, p. 397, 1 a and b.

- (A) The urine was allowed to collect in a vertical tube.
- (B) The outflow was blocked by clamping.
- (C) Fluid was injected through a T cannula, one end of its horizontal arm transmitting the pressure into the ureter, the other to a perpendicular glass tube to which was strapped a metre stick to facilitate the reading of pressure.

Almost without exception, in the experiments that were conducted without mishap, pressure up to 15 cm. did not cause elevation of the pressure recorded from the straight portion. The pressure in the pelvic portion almost invariably manifested a tendency to decrease, although under these conditions contraction waves were seldom recorded from the pelvis. When pressure higher than this

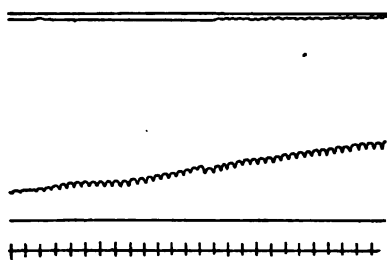


FIGURE 2. Lower tracing, time in seconds. Second tracing, base line for pressure in the straight portion of the ureter. Third tracing, pressure from the straight portion of the ureter. Fourth tracing, pressure from the renal pelvis. Fifth tracing, base line for pressure in the renal pelvis.

was exerted, a rise of pressure was frequently recorded from the straight portion, the contraction waves becoming often less frequent, sometimes more frequent, at which time the tendency to a higher pressure record from the L cannula was less pronounced. Even at this time the trocar, as a rule, recorded zero pressure in the renal pelvis. Nevertheless small, very rapid undulations frequently began to appear on the pelvic tracing (Fig. 2), and if the pressure was maintained for a time,

or if the ureter was subjected to deleterious conditions such as exposure to cold or desiccation, or if chloroform or any other muscular depressant was administered, the pelvic curve showed the tendency to development of a positive pressure, while the straight portion exhibited larger and larger waves, at less frequent intervals, on which smaller waves were often superimposed.

When, however, the pressure was permitted to continue for a sufficient time or increased, or if deleterious drugs were allowed to influence conditions, a sudden drop of pressure in the straight portion occurred synchronously with an abrupt rise in the pelvic pressure. Both pressures returned quickly to their previous levels, — the ureter pressure by short, step-like ascents, the pelvic pressure by shorter and more rapid descents (Fig. 3). The pelvic pressure



usually became neutral, the curves often entirely disappearing, the phenomena recurring again and again, tending to become more frequent; but recurrence was by no means regular. Quite often the large ureteral curves were not accompanied by the large pelvic curves.



FIGURE 3. Lowest tracing, time in seconds and base line for the pressure in the straight portion of the ureter. Second tracing, ureter pressure from straight portion. Third tracing represents the base line for the pressure curve of the renal pelvis. Fourth tracing, pressure from renal pelvis. The more rapid oscillations in the pelvic curve do not show in the figure.



FIGURE 4. Continuation of Fig. 3, thirty minutes later. Lower line, base line. Second tracing from bottom, from straight portion of the ureter. Upper tracing, from renal pelvis.

However, large pelvic curves never appeared under these conditions unaccompanied by decided increase in the size of the ureteral curves, but large pelvic curves could be induced previous to the appearance of, or unaccompanied by, the large curves of the straight portion, by rapidly infusing into a femoral vein 100 to 200 c.c. of warm physiological salt solution. Following the large pelvic curve thus produced, the curves of the straight portion, as a rule, became smaller and more frequent for a time, but subsequently the larger ascents and descents took place again. I am quite sure that the drops in pressure in the straight portion were due to fatigue and relaxation of the ureter; also that the synchronous rises in pressure in the pelvic portion, when they occurred, were due to reflux of urine into the renal pelvis, and the return to normal pressure brought about by the recovery and natural peristaltic action of the two portions.

When, however, the pressure was maintained or increased, or the action of deleterious agents brought to bear on the ureter, the large curves registered in the two portions became higher and the period of return to their previous levels was more prolonged. Subsequently, if the above-mentioned deleterious influences were continued, the

ureteral curves became more rapid, smaller, and regular. The pressure in the straight portion decreased and the tendency to production of large curves disappeared. The pelvic pressure increased, however, the curves recording it becoming larger and more regular. These curves from the two portions continued quite regularly for a long time, but occasionally a large ascending curve occurred in the ureteral tracing synchronous with a large descending curve from the pelvic tracing (Fig. 4). This, I believe, was not an evidence of further fatigue, but a manifestation of a tendency to recovery, and illustrates what I think occurs under normal conditions, but which I have been unable to demonstrate with my crude technique, *i. e.*, the tendency of the ureter, by its peristaltic action, to dispel or withdraw from the renal pelvis and kidney all pressure that would tend to arise from the collection of urine in the renal pelvis, perhaps even in the uriniferous tubules. Not infrequently, as before stated, a slight degree of negative pressure was recorded from the renal pelvis at the beginning of different experiments when the ureter was contracting actively. Such a negative pressure would explain also why, under my experimental conditions, no pressures could be recorded through the trocar connected with a water manometer, for the exertion of a counter suction caused by the column of water in the manometer would draw the ureter wall against the end of the trocar cannula and thus effectively prevent any variations of pressure from being transmitted.

It may be advisable to mention that when membrane tambours were used in place of water manometers, depressions in the pelvic curve were frequently noted to be synchronous with contractions of the straight portion of the ureter, even when fine undulations were not transmitted from the renal pelvis. It seemed, too, that the drop in pelvic pressure coincided closely (when inspected with the naked eye at the time the tracings were being recorded) with the longitudinal motion of the ureter.

**Analysis of some of the experiments on animals successfully used for study in situ of ureteral pressure.** — The phenomena produced under these experimental conditions, which appear to be constant and which, I believe, represent closely normal states, are the following: In four different animals there was a slight degree of negative pressure in the renal pelvis. At that time, in these experiments, the pressure in the straight portion varied from 0 cm. to 10 cm. of water, positive. In other experiments a negative pressure in

the straight portion of the ureter existed to the extent of 4 cm. The difficulty encountered in recording negative pressure from the renal pelvis was also met with in recording the pressure in the straight portion. However, the shape of the cannula may have furnished a condition slightly more favorable; its rigid half-cylindrical walls may have prevented the collapse of the ureter wall against the outlet tube to the manometer.

In two experiments showing positive pressure in the renal pelvis at the beginning of the experiment (in one, 8 cm., in the other, 6 cm. pressure) a kink in one of the tubes was subsequently detected which, on removal, allowed the pressure to fall and was without doubt the cause of the high pressure recorded. In four experiments neither negative nor positive pressure was recorded, nor were any contraction waves transmitted until positive pressure was caused by infusion or blocking the outflow of urine. These results demonstrated that the connections and ureter were patent. I am inclined to believe that the above-mentioned neutrality of pressure was due to the fact that the contractions acted so efficiently in carrying the urine away from and past the cannulas that there was lacking the minimal positive pressure necessary for the production of curves.

As a rule, increased pressure in the renal pelvis, whether caused by rapid infusion of physiological salt solution into a large vein of the animal, or by injection with a syringe through the wall of the rubber tube connection between the trocar and manometer, promptly increased peristalsis. The latter increased directly as the pressure, shortly afterward regaining the previous frequency and extent. The fact that in the straight portion the peristaltic rate was not so great, even when the pressure in this portion was still rising, *after* the injection of solution had been discontinued, suggests that the flow of fluid along the ureter under such conditions may also tend to act as a stimulus to the contractions aside from that induced by its distention.

Although as much as 500 c.c. of physiological salt solution was infused, in 100 c.c. amounts, at intervals of thirty minutes or less, into dogs weighing about 25 kilos, the ensuing increased flow of urine was very marked, but only a temporary rise of pressure in the renal pelvis and straight portion was noted. There was, of course, an immediate rise of blood pressure. In the renal pelvis the pressure increase occurred simultaneously with the infusion, disappearing very shortly after the infusion was stopped. Rise in

the straight portion was somewhat slower, higher, and of longer duration. The usual stimulation of ureteral contractions during the increase of pressure was noted, and it was found that, without exception, the pressure quickly returned to the one recorded previous to the infusion. In fact, the saline infusions, under conditions where the resistance to the outflow was not too great, seemed to favor peristalsis more decidedly and to cause a diminution of intra-ureteral pressure.

**Conclusions.** — I believe these experiments indicate very strongly that under normal conditions the intra-ureteral pressure remains at zero. The amount of urine in the ureter that ordinarily is necessary to call forth peristalsis is probably so slight that it causes scarcely any pressure in the relaxed ureter, and in the case of normal peristaltic contraction directs the urine into the bladder. At the same time a tendency to the production of negative pressure is doubtless exerted behind the mass of urine that proceeds downward. The flaccid muscular walls would be collapsed by such a negative intra-ureteral pressure, however. A mechanism by which negative pressure could be attained to an appreciable degree in the straight portion is difficult to conceive. In the renal pelvis conditions are somewhat different. In the first place the shape of the ureter above the isthmus becomes more and more flared. This portion is held open by its attachment to the firm kidney substance. Again this portion is abundantly supplied with nerves. From my observations of the action of this portion of the ureter, I am inclined to believe that here conditions prevail which are less favorable to accumulation of urine and more favorable to a negative pressure. However, there are other phenomena than those already mentioned that indicate highly co-ordinated and purposeful actions of this portion.

From the above observations, based as they are on experimental study, it appears that the ureter is specially antagonistic to transmission of pressure towards the kidney. The portion of the ureter above the middle, unenervated third, is apparently even more efficient in its resistance than the lower portions. The pressure necessary to overcome this action, under the above-mentioned conditions of experimentation, is somewhat above that produced by a 30 cm. column of water, and varies naturally with the duration of application, drugs used, exposure, size of animal, etc.

### III. URETERAL PRESSURE AND RENAL CIRCULATION.

**Introductory.** — The experimental results already recorded here indicate that the ureter is more than a simple conducting tube. It not only carries urine away from the kidney and discharges it into the bladder, but by its peristalsis it also prevents collection of urine in the renal pelvis, thus inducing a condition favorable to continuous flow from the tubules and spaces of Bowman's capsules. This state of affairs brings to mind a much studied problem, namely, the nature of urinary secretion.

The physical phenomena in the secretion of urine have recently been extensively studied by Sollmann,<sup>18</sup> who states with accuracy, "a knowledge of the mechanical phenomena occurring in the kidney would seem to be a necessary prerequisite to the discussion of any theory of urine secretion."

It is obvious that any influence the ureter may exert on the kidney must be primarily mechanical. Therefore I have made a study of certain mechanical phenomena in urinary formation, with the ureter and the glomeruli of the kidney especially in mind as influencing factors.

**Experimental. Methods.** — In these investigations I have followed in a general way Sollmann's technique for perfusing excised kidneys. I am indebted to Professor Sollmann for important suggestions in this connection. My experiments differ chiefly from his in that most of his work was done on kidneys in which the vital action was very largely excluded and the action of the ureter given little attention, while I have tried to control the mechanical phenomena to as large a degree as possible by removing the kidney and ureter from the animal, at the same time endeavoring to maintain the vital action of the ureter.

I have also performed a number of perfusion experiments by Sollman's technique exactly, in order to test his methods. Finding that my results confirmed his observations and conclusions, I based many of my tests and observations on his analogous data.

*The vitality of the kidney and ureter is very persistent.* — Sollmann presented evidence showing that a certain degree of vitality is maintained by the excised kidney for many hours. His experiments and conclusions applied to both the vitality of the se-

<sup>18</sup> SOLLMANN: This journal, 1905, xiii, p. 241.

cretory epithelium and the blood vessels. His results for the vessels are classified under the heads: (1) Dilator reaction; (2) Adrenalin reaction; (3) Hydrocyanic acid reaction. Sollmann's experimental results show that the vessels maintain vital activity to some degree two days after excision.

As I stated before, my attention has been directed in this research to the vascular system of the kidney and to the ureter. I have confirmed the results of Sollmann on the vessels, and have investigated, in addition, the effects of the following on the ureter and on the vessels of the kidney: adrenalin, barium chloride, caffein, diuretin, chloral, chloroform, magnesium sulphate, physostigmin, atropin, pilocarpin, cocain, sodium chloride, oxygen, carbon dioxide, cerium nitrate, heat, cold, mechanical irritation and electricity.

My observations of the effects of the above-named substances and conditions will be taken up in detail in the fifth section of this paper. It is sufficient to state here that the vessels of the excised kidney and the ureter are susceptible to the influence of drugs for many hours after excision.

In the living animal there are many conditions that influence the renal circulation. The following technique was used to eliminate such undesirable influences as changes in general blood pressure and nervous control.

The animal was anesthetized with ether or the skin over the femoral artery cocainized, the artery exposed and cannulized, and the animal bled to death. An incision was then made along the linea alba from ensiform cartilage to symphysis pubis and, by transverse cuts, extended from the first wound through the abdominal muscles to the dorsal region, passing just below the last rib. The ureter, kidney, and bladder were exposed by retracting the other abdominal viscera in warm towels. The artery of the kidney to be studied was exposed and cannulized with as large a glass cannula as could easily be inserted. This was filled immediately with warm Ringer solution, and, by means of a ten-ounce hand syringe joined to the cannula by a short piece of rubber tubing, the kidney vessels were flushed with Ringer solution at 38° C. until the vein flow was seen to be clear. The vein was then cannulized. A trocar cannula was introduced and retained in the renal pelvis, and an L cannula inserted into the straight portion of the ureter. If the ureteral flow was to be noted or the effect of injection into it studied, a small straight glass cannula was inserted just above the bladder

and the ureter severed below the cannula. The kidney and adjacent vessels and cannulas, with the ureter, were removed to a bath of Ringer solution maintained at a constant temperature of  $38^{\circ}$  C.; the artery was connected to a perfusion apparatus consisting of a 20-litre bottle, filled with Ringer solution and fitted with a Mariotte tube for maintaining constant pressure, and elevated to such a height that an injection pressure of 122.5 cm. was constantly maintained. A litre flask was inserted in the system and placed in a water bath, by means of which the temperature of the injection fluid could be kept constant. A thermometer inserted through the vertical arm of a T tube just proximal to the point of injection indicated the temperature of the perfusing fluid at its entrance to the artery, which temperature was maintained at  $38^{\circ}$  C. The cannula in the straight portion of the ureter and the one in the renal pelvis were each connected to a membrane tambour. This tambour was adjusted to write on the revolving smoked drum of a kymograph on which the time was marked in seconds, and the rate of vein flow recorded in drops from the vein cannula allowed to fall on the paddle of a Marey tambour. The outlet cannula of the ureter was connected by means of a T tube to a perpendicular tube by which the rate of flow or the resistance of flow to artificial pressure could be varied at will.

**Results.** — The fifth experiment being representative, it will be used as illustrative of the phenomena thus far observed by this method. In this experiment the outlet cannula of the ureter was connected with the perpendicular tube for the purpose of causing automatic increase of pressure at the distal end of the ureter.

5. February 18, 1908. — On opening the arterial clamp the kidney became tense, the vein flow started immediately and was very free — twelve drops per second. The pressure in the renal pelvis rose abruptly as the injection fluid entered the kidney. The pressure was slight and showed no tendency to change for several hours. No contraction curves were registered from this portion of the ureter until later, when the straight portion was overcome by pressure and fatigue.

The pressure in the straight portion of the ureter remained at zero for ten minutes, no peristaltic waves being recorded. At the end of that time a very slight rise in the intra-ureteral pressure of the straight portion was noted, when the peristaltic waves began abruptly and continued at the rate of one in ten seconds, tending

to increase in rate as the pressure rose, until the pressure reached 18 cm., when the curves became larger and slower.

After the rapid primary flow from the vein had reached its maximum there was a gradual decrease as the time of perfusion advanced. In what Sollmann<sup>10</sup> describes as the third stage in perfusion of the excised kidney, at the point where the vein flow remains almost constant (Fig. 5), there was a tendency at times when the ureter action was most efficient, toward an increase in the vein flow (Fig. 6).

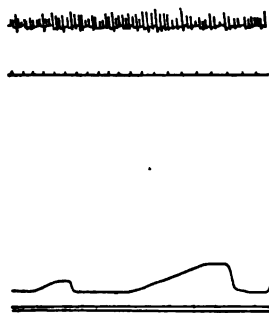


FIGURE 5. Upper tracing, vein flow in drops. Second tracing from top, time in seconds. Third tracing from top, ureteral pressure through the cannula in the middle of the straight portion. Fourth tracing from top, pressure from renal pelvis. Lowest tracing, base line for both pressure curves. The tracings were recorded two hours and ten minutes after perfusion was started. The number of ureteral contractions during the last thirty minutes of this time averaged 56.1 per ten minutes; the vein flow per ten minutes, 2056.8 drops (124.4 c.c.)



FIGURE 6. Same as Fig. 5, obtained twenty minutes later, the resistance to the ureteral outflow having been increased in the meantime from 5 to 10 cm. of water. Ureteral contractions averaged 109 per ten minutes. Vein flow, 3840 drops (228.5 c.c.) in the same time.

When relaxation and inactivity of the ureter took place, there was a marked decrease of the pressure in the straight portion of the ureter which was transmitted to the pelvic portion; at the same time the vein flow was abruptly reduced from 10 to less than 1 drop per second (Fig. 7). When the intra-ureteral pressure was rapidly increased by injection of Ringer solution through the outlet cannula, the primary effect of the increasing pressure in the renal pelvis was an increased vein flow (probably due to expression from the renal vessels). The vein flow then steadily decreased as the pressure increased. When a maximum pressure of 38 cm.

<sup>10</sup> SOLLMANN: This journal, 1905, xiii, p. 249, Fig. 2.



was caused in a period of twelve seconds, the above-described phenomena were noted in spite of the maintenance of this maximum pressure, and the vein flow again tended to increase rapidly, but in the succeeding ten minutes did not attain the rate noted previously to the inauguration of the pressure (Fig. 8). Again, if the above-mentioned pressure was obtained by six separate injections at intervals of ten to fifteen seconds, the retardation of the vein flow was much less distinct.

#### IV. URETERAL PRESSURE AND THE FLOW OF URINE.

**Introductory.** — The results of many of my experiments have led to the conclusion that the ureter acts in an antagonistic manner to pressures toward the kidney, and that this action of the ureter not only protects the kidney from pressure caused by accumulation of urine in the renal pelvis, but also encourages the flow of blood throughout the kidney.

Inasmuch as the ureter influences by its action or non-action the flow of blood throughout the kidney, the ureter must, to that extent at least, influence the flow of urine. In some of my experiments I observed that, in the excised ureter and kidney, the ordinary relation between them was somewhat different from any noted when *in situ*. The present study has been carried out on the kidney and ureter in their normal relationships in order to determine more intimately, if possible, the ureteral influence on urinary flow.

**Methods.** — The animal was kept on a soft or liquid diet for several days, then narcotized by hypodermic injection of morphine, placed in a dog-holder,<sup>20</sup> and shaved dorsally from the last rib to the crest of the ileum on each side. An incision was made from the angle between the last rib and vertebral column, extended down and outward, and the ureter in its middle or lower third was exposed retroperitoneally. A small glass cannula was introduced into the ureter, which was severed below the cannula and brought to the edge of the wound, the wound closed by interrupted sutures, and the ureter fixed by attaching it, by a slight stitch in its wall, to the surrounding muscle. The urine from each ureter was caught in a 100 c.c. graduated cylinder and saved for examination. The normal rate of flow from each kidney was recorded. When this had been satisfactorily determined, one of the ureteral cannulas

<sup>20</sup> MEYER: This journal, 1907, xxix, p. 906.

was joined to a perpendicular glass tube of small calibre by the side of which a metre stick was fastened. The other cannula was not interfered with, nor was the ureter disturbed by the adjustment of connections. Thus the effect on the ureter and kidney of gradually increasing pressure caused by the secretory activity of a kidney, as well as the continuance of flow from each kidney, was observed.

Deductions regarding transmission of ureteral pressure to the kidney were made (a) from the aspect of the undulations brought about by the contractions of the ureter caused in the vertical tube



FIGURE 7.

FIGURE 7. Same as Fig. 5, four hours after perfusion was started. Peristalsis infrequent and irregular, ureter relaxing at times, allowing pressure to be transmitted to the renal pelvis. Vein flow averaged 1510 drops (94.25 c.c.) per ten minutes.

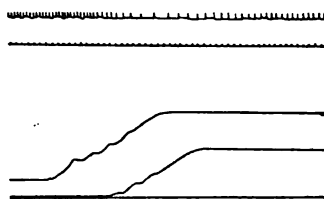


FIGURE 8.

FIGURE 8. Same as Fig. 7 (later). Peristalsis having ceased, ureteral pressure rapidly increased to 38 cm. (of water).

through which the pressure was exerted; (b) from changes in the amount and composition of the urine, and (c) from the results of *post mortem* examination of the kidney.

The cannulas were maintained in the ureters two or three days, at the end of which time they ceased to remain in place, because of pressure necrosis of the ureter due to the retaining ligature at the point of insertion into the ureter. The animal was continuously protected from pain by small though sufficient doses of morphine. Readings were made on each day for periods of three to five hours.

Inasmuch as the results obtained on the first days of Experiment No. 2 are typical, they will be cited by way of illustration.

**Results.** — The curves of Fig. 9 show two distinct effects of pressure on the rate of flow, *i. e.*, a decided increase between pressures of 2.5 and 25 cm., and a decrease commencing as the pressure rose above the 25 cm. mark. At the time of experimentation it was noted that while the flow was increased as the pressure rose in the perpendicular tube towards the 25 cm. mark, the meniscus rose and fell in a rapid and regular manner, which fluctuations, had the

pressure been recorded by the graphic method, would have given a curve of the character shown in Fig. 2 (third tracing from the bottom). I believe the intra-ureteral pressure conditions represented by the figure referred to are entirely analogous to those attained by the above-mentioned method, *i. e.*, the pressure exerted at the ureteral outlet is not transmitted to the renal pelvis, therefore does not act on the kidney, as such, although the stimulation of the ureter may have an indirect effect on the kidney.

When the column of urine nearly reached the 25 cm. mark, the oscillations became greater in extent and irregular in rate. The same pressure also caused retardation of the flow of the urine. The oscillations of pressure, if recorded at that time, would have exhibited a curve of the type shown in Fig. 3 (second tracing from the bottom). The oscillations indicated, I think, the time at which the ureter muscle was succumbing to the pressure and fatigue. At that point, for the first time, pressure as such exerted at the outlet of the ureter was transmitted through the ureter to the kidney. I do not believe that even at that time the pressure was transmitted in its entirety. Furthermore, its action was probably only for brief periods.

The column of urine continued to rise and oscillate in the above-mentioned manner until, after a time, the pressure approached 50 cm., when again the type of oscillation of the column of urine gradually changed to that represented by the curve in Fig. 4. This curve is characterized by the sudden falls of pressure and the more gradual returns to the previous levels, by very small and rapid oscillations, as shown by the second curve from the bottom of Fig. 4.

As the pressure increased from 50 to 67 cm. the fluctuations became smaller, being of the type shown on the ascending portion of the curve in Fig. 3. As the pressure gradually rose during this period, fluctuations of greater extent occasionally appeared, as is shown in Fig. 4.

After the column of urine reached the 65 cm. mark, there was no further ascent during the remaining thirty-six minutes of observation, the oscillations in the pressure tube also disappearing.

Without exception, in these experiments slight pressure, *i. e.*, from 2.5 to 25 cm., was accompanied by increased flow of urine from the ureter in which the pressure was exerted. The results of previous experiments on the ureter, compared with the oscillations of the fluid in the pressure tube in these experiments, make

me certain that pressures exerted in the ureter between 2.5 and 25 cm. were not transmitted as such to the renal pelvis. It is probable, however, that pressure between 25 and 50 cm. may have been transmitted to a slight degree at moments of relaxation of the ureter, when the abrupt drops in the column were noticeable.

The behavior of the ureter, when the pressures varied between the 50 and 67 cm. marks, suggests that its resistance was overcome, and that such pressures were transmitted almost entirely to the kidney, under which condition there was absolute stoppage of the urine flow from that kidney during a period of eighteen minutes.

The animal was then returned to its cage at 4.30 P. M. (October 12, 1907), and given food and water (500 c.c.).

The curve plotted from the rate of ascent of the urine secreted in the vertical tube is shown in Fig. 9.

The total amount of urine excreted between 4.30 P. M. (October 12, 1907) and 9 A. M. (October 13, 1907) was 175 c.c., at which time the animal was given 500 c.c. of water. At 10 A. M., 0.044 gm. of morphine were injected hypodermically. At 10.45 A. M., 350 c.c. of clear material was vomited. When the animal became thoroughly narcotized, it was again placed in the dog-holder, and at twelve o'clock the collection of urine was begun. The urine from the left kidney was clear and reddish, while that secreted by the right kidney was clear and yellowish.

The rate of flow from each kidney was recorded as follows:

Time of observation.	Urine from right kidney.	Urine from left kidney.	Remarks.
	c.c.	c.c.	
First 30 minutes	6.20	6.15	The urine was allowed to flow from the ureteral can- nulas without resistance.
Second 30 minutes	4.80	4.85	
Third 30 minutes	4.00	4.00	
Fourth 30 minutes	2.90	....	The ureter from the left kidney was attached to a ver- tical tube; the right, as above.
Fifth 30 minutes	2.20	3.50	
	No albumin. Sp. gr. 1.0245	Albumin present. Sp. gr. 1.0277	

## V. BIOCHEMICAL INFLUENCES ON URETERAL PRESSURE.<sup>21</sup>

The ureter is a highly specialized, involuntary muscular organ, and has been the fruitful subject for many investigations of the myogenic and neurogenic origin of automatic muscular contractions.

<sup>21</sup> Some of the experiments of this section were performed in the Department of Pharmacology in this institution under the direction of Dr. A. N. Richards, to whom I am indebted for much assistance.

The conclusions of such studies of the ureter have often been applied to the beating of the heart and to the movements of the intestines and other organs largely made up of smooth muscle fibres. However, the extent of the nerve supply of the middle portion of the ureter is a debatable question (Englemann, Dogiel, and others).

Protopow made an extended study of the separate existence of the requisite elements for muscular contractions.<sup>22</sup> He used the ureter as the subject of his investigations, which were both histological and biochemical in nature. He concluded that the requisite elements for muscular movements are found separately in the ureter of man and the higher animals. He also stated that stimulating the splanchnic

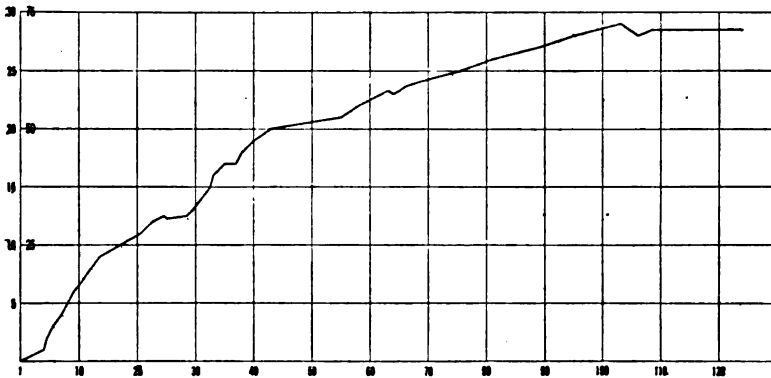


FIGURE 9. The curve is constructed from the records of the time required for the excreted urine to make successively an advance of one inch in the vertical tube; increasing pressure is automatically exerted by the rising column.

nerves has a motor effect on the ureter. Fagge<sup>23</sup> pointed out that stimulation of the hypogastric nerve has a motor effect on the portion of the ureter adjacent to the bladder.

Of more purely biochemical nature are the researches of Stern,<sup>24</sup> Hedon and Fleig,<sup>25</sup> Manevitch,<sup>26</sup> Pugliese,<sup>27</sup> and others, in which the control of automatic movements of the ureter by various cations and anions has been extensively studied. Hedon and Fleig investigated especially the effects of the ions which are found in the various artificial blood sera.

<sup>22</sup> PROPOW: *Archiv für die gesammte Physiologie*, 1897, lxvi, p. 1.

<sup>23</sup> FAGGE: *Journal of physiology*, 1902, xxviii, p. 304.

<sup>24</sup> STERN: *Thèse de Geneva*, 1903.

<sup>25</sup> HEDON et FLEIG: *Archives internationales de physiologie*, 1905-1906, iii, p. 95.

<sup>26</sup> MANEVITCH: *Revue médicale de la suisse romande*, 1907, xxvii, p. 585.

<sup>27</sup> PUGLIESE: *Archives italiennes de biologie*, 1906-1907, xliii, p. 54.

Manevitch divides the cations which affect the contractions of the ureter into three groups: (1) Those which have the power of preserving to the highest degree the automatic contractions of the excised ureter (smooth muscle tissue), *e. g.*, Na and Li; (2) Those which depress the tonus and stop the rhythmic and automatic function of smooth muscle, K,  $\text{NH}_4$ , Mg, Zn, Cd, Pb, Co, Ni, Fe, Mn, Cu; (3) Those which are stimulants to the tonus, and aid development of the rhythmic and automatic action of smooth muscle, foremost among which are Ba and Sr. Ca, according to Manevitch, occupies a special place, having some of the characters of barium and strontium. It causes a development of deficient automatic contractions, and renders better, and more energetic, the contractions already in progress, but, on the other hand, often tends to inhibit or retard the rhythm.

The cations Sr and Ba are conceded by Manevitch to be antagonistic, in their action on smooth muscle tissue, to those of the second group named above, *i. e.*, to K,  $\text{NH}_4$ , Mg, etc. Manevitch also states that when the automatic contractions of the ureter have become greatly diminished after hours of action in solutions containing indifferent cations such as Na, Li, Cl, they are again greatly revived by solutions containing Ba or Sr cations.

From a study of the literature, one sees that the activity of the ureter must be greatly influenced by chemical as well as nervous influences. It seems probable, then, that a study of the influence on the ureter of chemical substances which occur as normal constituents of blood and urine compared with the effects of substances appearing in these liquids when used as drugs that act on the kidney (and ureter?), may give us information concerning both the function of the ureter and the action of drugs. The specific influence of drugs on the ureter in a normal animal cannot be precisely controlled. However, their influences can be determined by excising the organ and placing it in one of the artificial blood sera whose action has been definitely ascertained. After such a determination the influence of drugs under ordinary conditions can be satisfactorily recognized with due regard for other vital processes affected by them.

**Experiments on the excised ureter. Method.** — Usually a cat or small dog was chosen, which was anesthetized with ether, or bled to death. The portion of the ureter to be studied was quickly isolated and removed to a bath of warm physiological salt solution, where

the adjustments of such cannulas and apparatus as were to be used in the particular experiment were made, one of the three following methods of procedure being used:

(A) The isolated piece of ureter was ligated at both ends, one end anchored at the bottom of the bath, the other to a writing lever which traced on a smoked drum.

(B) The anchorage of the ureter was attained by inserting the end of a curved glass cannula into the lumen of the lower end, through which warm physiological salt solution was injected for the production of any desired intra-ureteral pressure. The effects of the contractions were not only recorded on the drum as in method A, but could also be noted in the fluctuations of the fluid in the vertical pressure tube.

(C) The ureter was placed horizontally in the bath, a cannula inserted in each end as in method B, and a myocardiograph attached to the ureter as for recording contractions of the heart.

The effect of *barium chloride* on the ureter is illustrated by Experiment I, which was performed by technique A.

1. October 27, 1907. — Medium-sized cat, killed by decapitation. The middle third of one ureter and a section of the small gut of the same length as the section of ureter were excised, ligatures tied to the ends of each, the sections anchored at the bottom of a beaker containing Locke solution at 40° C. (through which oxygen bubbled constantly), and the free end of each attached to a spring writing-lever which traced on a revolving smoked drum.

The gut began to record contractions immediately, the ureter remaining perfectly motionless for forty minutes, at the end of which time 1 c.c. of 5 per cent barium chloride was added to the 40 c.c. of Locke solution contained in the bath. The intestinal contractions were immediately increased greatly. The ureter made its first contraction thirty seconds after the addition, which was followed by contractions occurring every twenty-five to thirty seconds and showing for a short time a tendency to become stronger. Sometimes the excursion of the recording arm was very great, the succeeding pause being correspondingly lengthened. Later there was a tendency for two contractions to occur in quick succession or for a second to occur before the complete relaxation from the first, the rate of contractions increasing, but the extent of contraction becoming less. As a rule, the period of rest following a double contraction was greater than that following a single contraction of equal extent. The contractions continued to become more fre-

quent and less in extent until they disappeared. Subsequent additions of barium chloride did not cause additional contractions.

The influence of *adrenalin* on the isolated ureter as tested by the same method was exhibited by a pronounced increase in tonus and contractility, but often there was no stimulation of the rate of contraction; in fact the number of contractions was often decreased, as shown in Experiments 2 and 3, protocols of which are appended:

2. December 6, 1907. — An ox ureter, obtained at a slaughter-house immediately after the animal had been killed by the usual method, was placed in a quart jar of Ringer solution at 38° C. and carried

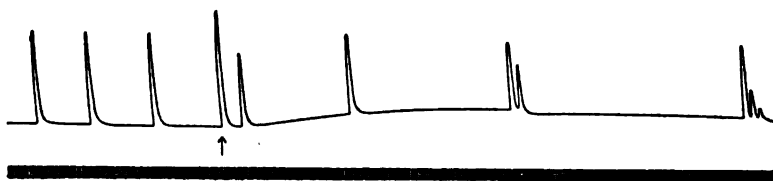


FIGURE 10. Lower tracing, time in ten seconds. Second tracing, contractions of the ureter showing the effect of adrenalin chloride.

to the laboratory, where it was subjected to the treatment of method B. The time that elapsed between the killing of the animal and the completion of all manipulations was about forty-five minutes.

During transportation of the ureter the solution in which it was immersed cooled to 35° C.

No contraction appeared during the first ten minutes after the application of method B, at the end of which time 1 c.c. of a 5 per cent solution of barium chloride was added to the bath of 500 c.c. of Ringer solution. Fifty seconds later the first contraction was recorded. It was followed by other contractions at gradually increasing intervals of from two seconds to two minutes. When the contraction rate had become one in about two minutes, 0.2 c.c. of 1:1000 adrenalin solution were added to the bath, whereupon the rate of contraction was distinctly decreased; but the line that was traced when the ureter was at rest gradually rose for fifteen minutes, regardless of the contractions which had begun to show a tendency to occur in groups of twos or threes (Fig. 10).

3. December 13, 1907. — Small dog. Etherized. A femoral artery was cannulized and the dog bled to death. The ureter was removed and placed in 500 c.c. of Ringer solution. Method B was



applied. No intra-ureteral pressure was exerted, and only one contraction took place during the first twenty minutes, which seemed to be the result of irritation caused by handling the ureteral cannula. The intra-ureteral pressure was then increased to 68 cm. Contractions immediately appeared, and recurred fairly regularly. The pressure was lowered to 50 cm., the extent of contraction becoming greater but the rate remaining about the same. The contractions continued regularly at this rate for forty-five minutes, when 0.2 c.c. of 1:1000 adrenalin solution was added to the bath of 500 c.c. of Ringer solution. The contractions continued at the previous rate for thirty-five seconds longer, then eleven contractions occurred in the succeeding thirty-eight seconds. After the last of these, no contraction occurred for twenty-five seconds, when a curve resulted which was composed of three contractions and reached a height about twice that of any previously recorded. This curve was followed by others of like character at the rate of one per minute, but after the first three of decreasing size and increasing rate, the curves showed an increasing tendency of the contractions to occur in groups (Fig. 11).

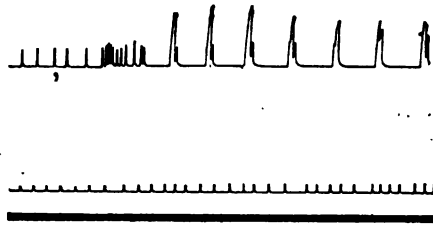


FIGURE 11. Lowest tracing, time in ten seconds. Second tracing, ureteral contractions by method *B* (where an intra-ureteral pressure of 68 cm. Ringer solution prevailed). Third tracing, intra-ureteral pressure had been reduced to 50 cm. at the mark (,) 0.2 c.c. of 1:1000 adrenalin solution was added to the bath of 500 c.c. of Ringer solution.

In other experiments with the same technique, where the contractions were very infrequent or entirely absent, a slightly larger dose of the adrenalin than those used in the experiments described in the preceding protocols caused a tonic contraction which did not show any tendency to relaxation after sixteen minutes, whereupon the bath was changed to plain Ringer solution. The muscle then gradually relaxed, returning in five minutes to its original state.

It seems, from the results of the experiments with adrenalin, that this substance increases both the contractility and the tone of the ureter muscle.

*Caffein* produced phenomena very similar to those caused by adrenalin. *Diuretin* also acted in a similar way. *Chloral*, *chloroform*, *ether*, and *magnesium sulphate* were distinctly depressant, showing at times a slight preliminary irritation.

In the experiments with the excised ureter by the method described above, *nicotin*, *atropin*, *muscarin*, and *physostigmin* gave only negative results (but I feel confident that these drugs exert definite influences and that they can be demonstrated graphically by improvement of the technique).

The ureteral contractions seemed to be developed less satisfactorily in oxygenated solutions than in unoxygenated ones.

The results of this study of the effects of drugs on the *excised* ureter warrant the following conclusions:

1. Adrenalin, *caffein*, and *diuretin* increase the tone and contractility of the ureter muscle.
2. Barium chloride increases the irritability more noticeably than the substances of the first group, and does not seem to have such pronounced influence on the tone, unless it is to depress it.
3. *Chloral*, *chloroform*, *ether*, and *magnesium sulphate* exert at first slight irritating action, but later cause marked depression.

These observations on the excised ureter cannot be exactly applied to the complete ureter, however, for the middle third was usually employed in these tests. Nerve influences would be much less prominent in this portion than in other portions.

**Experiments on the ureter in situ.** *Method.* — Dogs were used for all of these experiments. *Chloroform* or *ether* was employed only for the purpose of studying effects upon the ureteral peristalsis. The animal was narcotized with morphine, and the ureter exposed by an incision along the *linea alba* from symphysis pubis to ensiform cartilage. The abdominal walls were then retracted, the intestines were drawn to one side, and the viscera as well as the rest of the animal were covered with warm towels and cotton. The kidney was exposed by another incision along the lower border of the last rib, or by a small longitudinal incision directly over the kidney. The left kidney was usually selected on account of its lower and more accessible position.

Graphic representations of the ureteral movements were obtained as usual with a water manometer, the undulations of the column of water being transmitted by means of a float and style to a revolving drum. The connections with the ureter were made by two methods:

(A) By introducing into the ureter a cannula which is a modification of the Ludwig-Spengler artery cannula. With this cannula a much smaller incision than usual is required; no ligation being necessary, the propagation of the muscular wave of the ureter is only slightly interfered with, the nutrient vessels of the ureter can be avoided, and the nutrition of the ureter is only slightly impaired.

(B) By introducing a trocar through the kidney into the renal pelvis, and retaining it in place by a purse-string suture around the point of puncture of the capsule of the kidney. This also helped to stop bleeding, which, however, was surprisingly slight. A small quantity of warm salt solution or urine aspirated fresh from the bladder was injected through the needle; thus the patency of the cannula and ureter was ascertained.

In connecting the cannulas with the water manometer by means of narrow glass and rubber tubing, urine was separated from the water in the manometer by a column of air. Any movement of the urine caused an undulation in the manometer. These undulations were recorded on a drum by means of an Emerson float.

In most of the experiments of this series the ureter remained in normal connection with the bladder. In some experiments, however, the ureter was severed near the bladder, the urine escaping into the abdomen or being carried out of the body by a glass tube connected with the cannula in the ureter. This cannula narrowed, of course, the lumen of the ureter, and thus afforded some resistance to the flow of urine out of the ureter. In some of the experiments the urine was caused to drop on a pan connected with a Marey tambour, by means of which the flow of urine was recorded.

The dose of morphine varied from 0.06 to 0.12 gram, depending on the size of the animal. This was given subcutaneously sixty to ninety minutes previous to the operation. All experiments were commenced in the morning; the animals had not been fed since the previous evening, but they had free access to water.

The susceptibility of the ureter, *in situ*, to the various substances used in this study seemed to be much greater by this technique. *Chloroform*, administered in the respired air, caused marked decrease of both the extent and frequency of the contractions of the middle part of the ureter, and, if continued, completely abolished them. Sometimes, when the administration was brief, the deteriorating effect did not set in until a little while after the use of the anesthetic was discontinued. Shortly after recovery from the evil

effects of the chloroform in some of these cases, another period of deterioration set in as a second after-effect.

Frequently, when *ether* was suddenly exhibited in the respired air (inhaled *per nares*, not by tracheal cannula), a temporary change almost instantaneously appeared in the curve representing the peristalsis of the ureter in the renal pelvis. Sometimes entire cessation of the peristalsis occurred, which phenomenon could also be elicited by sudden irritation of the nostrils with a probe, — an observation very strongly suggestive of a reflex.

Moderate doses of *caffein* caused various effects in the different parts of the ureter, the portion in the renal pelvis regularly contracting in a somewhat tonic manner and causing thereby a very pronounced rise in the pressure for a short time in that part. This pressure appeared to be attainable through the agency of a sphincter-like action of the isthmus of the ureter, which prevented the urine from escaping. The pressure in the straight portion did not exhibit a simultaneous change.

*Adrenalin* also showed a tendency to disturb the normal pressure relations between the renal pelvis and the straight part of the ureter. It caused a very pronounced positive pressure, very much as *caffein* does.

*Barium chloride* seemed to stimulate the contractions of both the upper and lower portions without the same tendency to cause increased pressure in the renal pelvis.

When small amounts of *chloral* or *magnesium sulphate* were injected into the renal pelvis, only a direct depression was shown.

These tests, while very incomplete, show distinctly that the ureter is very susceptible to the action of drugs administered systemically as well as directly. The experiments with chloroform suggest that there may be a double action of drugs on ureteral muscular activity and tone. The first influence on the peristalsis was exhibited so promptly after the administration of chloroform had been begun, that it could hardly have been due to chloroform secreted into the urine in amounts sufficient to affect the ureter directly, although it seems possible that the circulating blood containing the drug might have some such effect. When the chloroform was withdrawn, at this early period, the very pronounced retardation sometimes did not appear until the animal gave indications that the general systemic action was wearing off, thus increasing the impression that drugs act on the ureter not only while circulating in the blood, but also when present in the urine.

From what I can find in the literature, together with impressions obtained in my own studies of the ureter, it seems that drugs which exert stimulating action on the ureter also appear to possess diuretic power to a somewhat similar degree. I think I am correct in saying that drugs which show a depressing action on the peristalsis of the ureter also often exhibit a tendency, when administered systemically, to decrease the amount of urine. These conclusions suggest that stimulation or inhibition of ureteral action may be a factor in the diuresis, or in the diminished flow of urine, caused by drugs having the above-mentioned influences. The solution of this problem presents a great many difficulties. Nevertheless it should be possible to gain some information regarding it by comparing the effects (on the volume of urine eliminated from each of the two kidneys with both ureters intact) of drugs whose influence is eminently diuretic and ureter-stimulating, *e. g.*, caffeine, or diuretin, with the flow of urine from each kidney after the ureter of one kidney has been completely eliminated.

This matter was tested in five experiments on dogs as follows:

*Effects of drugs on the comparative flow of urine.* — The animals were narcotized with morphine and the ureters exposed only at their entrance to the bladder. A small straight cannula was inserted into each ureter at this location, and the urine collected in small graduated glass cylinders. The normal flow was noted and recorded at regular intervals.

The flow from the kidneys of the same animal was found to be usually quite equal. Infusion of 150 to 200 c.c. of salt solution caused an average diuresis of 20 per cent from each kidney over a period of thirty minutes. The actual amount of diuresis varied in the different animals. No attempt was made to maintain uniform conditions in these animals previous to the experiment. The diuresis was usually quite equal from the kidneys of the same animal.

When, however, 1 gram of diuretin, dissolved in 50 c.c. of warm physiological salt solution, was infused in the femoral vein, the increase in urine from each kidney was equal in the same animal, but varied in different animals from between 250 to 300 per cent. After these preliminary tests had been made in each animal, the ureter from one of the kidneys was exposed at the renal pelvis, and a large glass cannula which flared out considerably at its end, so as to hold the portion of the ureter in the renal pelvis wide open, was inserted and retained by means of a ligature. Such a cannula

prevented any influence of the muscular contraction of the ureter on the flow from the renal pelvis and kidney. (Great care was exercised not to manipulate the kidney or interfere with the renal vessels.) The urine was conducted from the cannula into the graduated cylinder, care being taken to make certain that the degree of resistance to the flow of urine from each cannula was equal. This resistance varied from between 2 to 8 cm. in the different experiments, after all manipulation was completed. The rate of flow

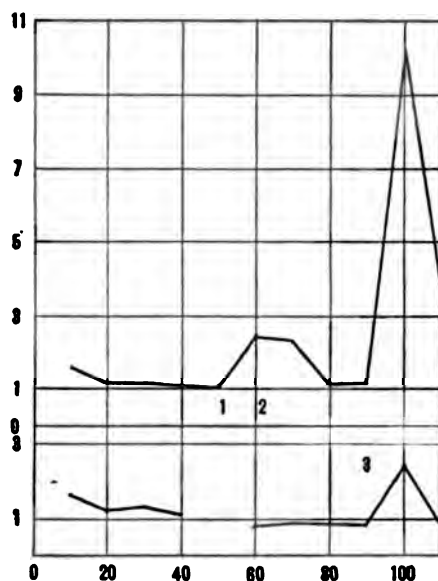


FIGURE 12. Upper curve gives the flow from the left kidney, lower curve that from right kidney. Cannula in renal pelvis. Ureteral action was removed from the right kidney at the end of 40 minutes of observation. The amount secreted was recorded at the end of each 10 minutes. (1) 50 c.c. salt solution. (2) 50 c.c. salt solution. (3) 1 gm. diuretin in 50 c.c. salt solution.

from each kidney was again observed, and as a rule a slight decrease in flow was noted from the kidney cannulized at the renal pelvis. Infusion into a femoral vein of 50 to 100 c.c. of physiological salt solution at this stage frequently failed to cause an increased flow from the cannulized kidney, while the flow from the kidney with the ureter intact showed in each experiment an increase of at least 200 per cent. After the flow from the kidney with its intact ureter had returned to the amount eliminated previous to the infusion, and the urine from each kidney was being excreted at a constant

rate, 1 gram of diuretin was infused in 50 c.c. of physiological salt solution. The average increase of the flow from the kidney with intact ureter was 800 per cent for the first ten minutes, falling to 200 per cent in twenty minutes. From the cannulized kidney there was only a 125 per cent increase in the first ten minutes with a return to the normal elimination in twenty minutes (Fig. 12).

Although the damage done by the manipulation when inserting the cannula into the renal pelvis cannot be overlooked as an influence tending to decrease the amount of urine excreted by that kidney, the above observations suggest very strongly a ureteral influence in the diuresis caused by drugs which increase the muscular tone and activity of the ureter.

## VI. SUMMARY OF GENERAL CONCLUSIONS.

I. If continued pressure in the bladder exerts a deleterious effect on the kidney, it does so by nervous influence and not by direct transmission of pressure from the bladder to the kidney.

II. Even under the artificial conditions of experimental study, the intra-ureteral pressure tends to remain approximately neutral in the various portions of the ureter. The ureteral pressure is surprisingly strong and efficient when called upon to maintain this intra-ureteral condition.

The effect of the antagonism of the ureter to pressure exerted in it must be carefully taken into account, especially in studies of the effects of artificial pressure through the ureter on the kidney.

The vital activity of the ureter is extremely persistent.

III. Collectively *excised* kidneys and ureters maintain sufficient vital activity, when the kidney is perfused with warm Ringer solution, to permit a study of the relation of the mechanical influence exerted by the ureter on the circulation of the kidney. Under these conditions the ureter is less susceptible to pressure influences. Therefore it is not so efficient in maintaining low-pressure conditions in the renal pelvis as when *in situ*.

Pressure in the renal pelvis lessens the circulation through the kidney.

*Sudden* increase in pressure in the renal pelvis shows more pronounced checking of the circulation than pressure of the same degree when *gradually* exerted.

Retardation of renal circulation by pressure exerted in the renal pelvis tends to be compensated for.

Ureteral peristalsis influences renal circulation and *vice versa*.

IV. Stimulation of the ureter by moderate pressure induces an increased flow of urine.

Pressure exerted in the renal pelvis diminishes the flow of urine.

A pressure of 67 cm. of urine acting in the renal pelvis causes distinct damage to the kidney, as shown by the presence of blood in the urine, and by the macroscopical appearance of the kidney.

V. There appears to be a ureteral influence in the diuresis caused by drugs which increase the muscular activity and tone of the ureter.

Professor William J. Gies made it possible for me to inaugurate my work on the ureter. Since that time he has never ceased to aid, encourage, and instruct me in research on this and other subjects. Whatever scientific or clinical advances have or may result from my efforts in research are directly dependent upon his interest and assistance.



# FURTHER EVIDENCE OF THE PRESENCE OF VASO-DILATOR FIBRES TO THE SUBMAXILLARY GLAND IN THE CERVICAL SYMPATHETIC OF THE CAT.

By F. C. McLEAN.

[From the Hull Physiological Laboratory of the University of Chicago.]

## I.

AFTER the discovery of vaso-dilator fibres to the submaxillary gland in the cervical sympathetic of the cat by Carlson,<sup>1</sup> it occurred to me that there was a further means of testing his results. Barcroft<sup>2</sup> has obtained results very similar to Carlson's. He, however, failed to obtain an acceleration in the rate of blood flow through the gland on stimulation of the cervical sympathetic, without a slight primary retardation. Elliott<sup>3</sup> has shown that the effect of adrenalin on any organ is the same as the effect of stimulation of the sympathetic nerve to that organ. If the results of both Carlson and Elliott be correct, the injection of adrenalin into the circulation should cause vaso-dilation in the submaxillary gland of the cat. Elliott has not observed vaso-dilation in the submaxillary gland from either stimulation of the sympathetic or from adrenalin. He was unable to show directly any vaso-dilation by adrenalin in any part of the body. But Dale<sup>4</sup> has observed a fall in blood pressure on injection of adrenalin after the vaso-constrictor action of adrenalin has been prevented by an injection of chrysotoxine. Elliott explains this on the assumption that there are sympathetic vaso-dilator fibres to some blood vessels, probably in the splanchnic region. Elliott failed to show vaso-dilation by adrenalin in the

<sup>1</sup> CARLSON: This journal, 1907, xix, p. 408.

<sup>2</sup> BARCROFT: Proceedings of the Physiological Society, 1907, p. xxix; Journal of physiology, 1907, xxxv.

<sup>3</sup> ELLIOTT: Journal of physiology, 1905, xxxii, p. 401.

<sup>4</sup> DALE: Journal of physiology, 1905, xxxii; Proceedings of the Physiological Society, May 20.

bucco-facial region of the dog, where Dastre and Morat<sup>5</sup> showed stimulation of the cervical sympathetic to have a vaso-dilator effect. He states, however, that stimulation of the cervical sympathetic in the animals he examined did not give any vaso-dilator effect. In my experiments I have, in the majority of cases, obtained a vaso-dilator effect in the submaxillary gland of the cat on injection of adrenalin.

## II.

The technique of the experiments was the same as that used by Carlson in his work. The blood flow through the gland was measured by allowing the blood to drop from the side tube of a three-way cannula placed in the external jugular vein after tying off all of the contributing veins except the one from the submaxillary gland. The other opening of the three-way cannula was attached to a sodium citrate bottle for washing out thrombi. The rate of blood flow through the gland was recorded in drops by means of an electro-magnetic signal. Adrenalin was injected in dilute solution (1-10,000) into the femoral vein in doses of 1-5 c.c. The duration of the injection was marked on the drum with a signal magnet operated by a spring key. When a simultaneous blood-pressure tracing was desired, it was taken from the femoral artery. The animal was kept under light ether anesthesia during the experiment.

## III.

In most of the experiments on the cat the injection of adrenalin was followed by a marked increase in the rate of blood flow from the gland. This could be due either to vaso-dilation in the gland, or to the rise in general blood pressure which adrenalin produces, the gland vessels remaining of the same calibre or being but slightly constricted. If the gland vessels were slightly constricted, we should expect a primary slowing, followed by an increase in the rate of flow as the vessels began to dilate again and the arterial pressure began to fall from the lowering of the resistance in the smaller vessels. If the gland vessels were not affected, we should expect an increased rate of flow while the blood pressure remained high,

<sup>5</sup> DASTRE and MORAT: *Comptes rendus de l'Académie des Sciences*, 1880, xcii, p. 393.

returning to normal with the return of the blood pressure to normal. If the gland vessels were dilated, we should expect a primary increase in the rate of flow, more rapid when the blood pressure is

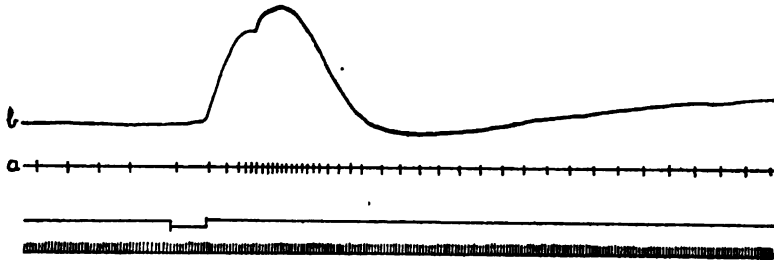


FIGURE 1.—(a) Record of blood flow (in drops) from the submaxillary vein of cat on injection of adrenalin. Showing vaso-dilator effect. (b) Blood pressure from femoral artery. Time, seconds.

high, but not necessarily returning to normal with the return of normal blood pressure. The latter effect was noted in most of the experiments (Fig. 1). In a few experiments, however, we obtained a primary slowing, followed by an increased rate of flow during relaxation of the blood vessels and fall of general blood pressure

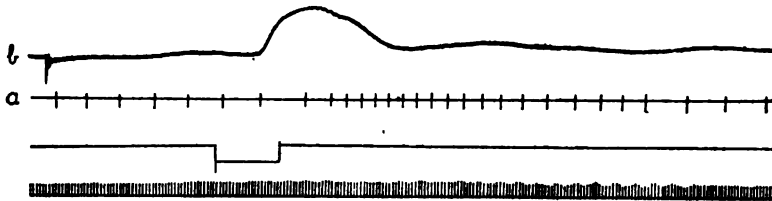


FIGURE 2.—(a) Record of blood flow (in drops) from submaxillary vein of cat on injection of adrenalin. Showing primary vaso-constrictor action. (b) Blood pressure from femoral. Time, seconds.

(Fig. 2). This agrees with the work of Carlson, who found that in a certain number of cases stimulation of the cervical sympathetic caused only vaso-constriction. In the cases where we observed vaso-constriction from adrenalin the same result was noted on stimulation of the cervical sympathetic.

For purposes of comparison a few experiments were carried out on the parotid of the cat and on the submaxillary of the dog, as stimulation of the sympathetic to these glands is known to cause vaso-constriction. In these cases an acceleration of short duration was usually noticed on injection of adrenalin, followed by a long period of slowing. The primary acceleration was probably due

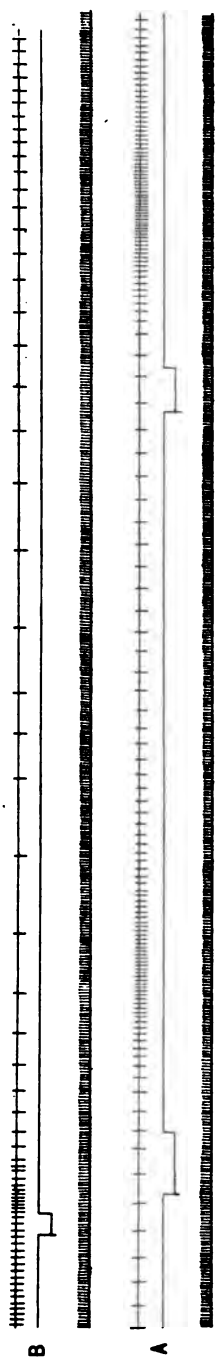


FIGURE 3.—Record of blood flow (in drops) from (a) the submaxillary vein, and (b) the parotid vein of cat on injection of same amount of adrenalin. Showing the opposite vaso-motor action of adrenalin on the two glands. Time, seconds.

either to the rise of general blood pressure before the gland vessels were affected or to the squeezing out of the blood already in the gland vessels by their constriction. The secondary slowing was due to the blocking of the passage of blood through the gland by the constriction of the vessels. This result is quite the opposite to the result on the submaxillary of the cat, where, as a rule, no slowing was observed. Fig. 3 shows a comparison between the action of adrenalin on the vessels of the submaxillary and parotid of the cat. The same results were obtained from the submaxillary of the dog as from the parotid of the cat.

Another result was sometimes obtained by Carlson, which was observed in these experiments in some cases. This was a periodic variation of the dilator action. During the injection of the adrenalin an acceleration of the flow through the gland was noted, followed by a return nearly to normal before the injection was stopped. Following this there was a second acceleration for a short time.

These experiments were made in August, 1907, but have not been recorded before, as I have been waiting to obtain some chrysotoxine, which so far has not been obtained. By giving chrysotoxine before adrenalin the factor of the general blood pressure in the rate of flow from the gland might be eliminated, and the true effect of the adrenalin on the gland vessels could probably be noted. If there are some vaso-dilator fibres in the sympathetic of the dog to the parotid and submax-

illary, and to the parotid of the cat, as Carlson suggests, we should be able to show their presence by cutting out the vaso-constrictors by chrysotoxine and stimulating the vaso-dilator apparatus by adrenalin, as chrysotoxine seems to affect the vaso-constrictors and not the vaso-dilators.

# ON THE AVAILABLE ALKALI IN THE ASH OF HUMAN AND COW'S MILK IN ITS RELATION TO INFANT NUTRITION.

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ON account of its importance as a food and the interest attaching to it as an animal secretion, and in consequence of the numerous attempts which have been made from time to time by various observers to account for the differences observed in the nutrition of breast-fed infants and those artificially fed upon cow's milk, the composition of milk, especially that of cow's milk and human milk, has been the subject of a large number of investigations. From the results of a large number of analyses of human and cow's milk by Pfeffer, Ruebner, Koenig, Leeds, Harrington, Adriance and others, Holt<sup>1</sup> gives the following compilation as representing the average composition of human and cow's milk:

	Human milk. Per cent.	Cow's milk. Per cent.
Fat . . . . .	4.00	4.00
Sugar . . . . .	7.00	4.50
Proteids . . . . .	1.50	3.50
Salts . . . . .	0.20	0.75
Water . . . . .	87.30	87.25
	100.00	100.00

Lehmann<sup>2</sup> has pointed out that the discrepancies so frequently encountered in the literature regarding the composition of human

<sup>1</sup> HOLT: Diseases of infancy and childhood, Appletons, 1908.

<sup>2</sup> LEHMANN, JULIUS: Milk investigations of, WALTHER HEMPEL, Archiv für die gesammte Physiologie, 1894, lvi, pp. 558-578.

milk are to be explained by the fact that some observers have analyzed the first milk obtained from the gland, whereas others have analyzed the later portions. According to this author the first portions of the milk are poorer and the last portions richer in fat. He therefore gives the following results of his own determinations of the composition of human and cow's milk, in which the whole milk was analyzed in each case:

	Human milk Per cent.	Cow's milk. Per cent.
Fat . . . . .	3.8	3.5
Sugar . . . . .	6.0	4.5
Casein . . . . .	1.2	3.0
Albumin . . . . .	0.5	0.3
Ash . . . . .	0.2	0.7
Water . . . . .	88.3	88.0
	100.0	100.0
Total solids . . .	11.7	12.0

The essential points of resemblance and difference thus far made out, therefore, between human and cow's milk may be briefly summarized as follows: Both are complex fluid mixtures containing approximately the same amount of water, solids, and fat, and both yield on combustion practically the same amount of heat per kilogram of each consumed, — in round numbers, 700 calories per kilogram; both contain certain formed elements, deriving from the blood and mammary glands of the animal, and both contain certain characteristic enzymes, and both exhibit certain biological properties in common.

The ash of the two kinds of milk contains the same elements in all probability in essentially similar combinations. Both contain relatively the same amounts of chlorides and magnesium, and approximately the same amount of sodium. For analyses of the ash of human and cow's milk, see Tables IV and V.

On the other hand we find that cow's milk contains about 3.3 per cent of proteid, whereas human milk contains only 1.7 per cent; cow's milk contains about 4.5 per cent of carbohydrate, while human

milk contains 6.0 to 7.0 per cent; cow's milk contains 0.7 per cent of ash, whereas human milk contains only 0.2 per cent.

According to Williams,<sup>3</sup> the fat of human milk contains a very high proportion of unsaturated fatty acids compared with cow's milk, in consequence of which it is, according to this author, more readily absorbable.

According to Soxhlet,<sup>4</sup> cow's milk contains four times as much phosphoric acid and six times as much lime as is contained in human milk. Human milk contains about one third more potassium than cow's milk, which is of considerable interest in view of the fact that the ash of the infant contains such small amounts of this element. Human milk also contains a considerably larger quantity of iron than cow's milk. Further, certain differences have been noted by Lehmann<sup>5</sup> in the amounts of sulphur and phosphorus contained in the casein of the two kinds of milk, and certain physical differences have been observed in the general character of the curd resulting from the action of rennin on the two kinds of milk. Finally, certain differences have been observed with respect to the amounts or activity of certain of the ferments contained in the two kinds of milk, and also certain differences in their biological properties. According to Béchamp,<sup>6</sup> for example, human milk contains an active diastase, whereas cow's milk either contains no diastase or, at any rate, it is much less active than the diastase of human milk, and Moro<sup>7</sup> has observed that while human milk rapidly coagulates hydrocele fluid, cow's milk does not possess this property. Gillet<sup>8</sup> and more recently Kastle and Porch<sup>9</sup> have observed that cow's milk exhibits greater peroxidase activity than human milk towards a considerable number of peroxidase reagents.

These, in the main at least, are the most essential points of resemblance and difference that have thus far been made out relative to the composition and properties of these two kinds of milk.

In this connection it is interesting to note that of all milks of dif-

<sup>3</sup> WILLIAMS: Bio-chemical journal, 1907, ii, p. 406.

<sup>4</sup> SOXHLET: Münchener medicinische Wochenschrift, 1893, xl, pp. 60-65.

<sup>5</sup> LEHMANN: *Loc. cit.*, pp. 576-577.

<sup>6</sup> BÉCHAMP: Comptes rendus, 1883, xcvi, pp. 1508-1509.

<sup>7</sup> MORO: Wienerklinische Wochenschrift, xv, pp. 121-122.

<sup>8</sup> GILLET: Journal de la physiologie et de la pathologie générale, 1902, iv, pp. 503-518.

<sup>9</sup> KASTLE and PORCH: Journal of biological chemistry, 1908, iv, pp. 301-320.



ferent animal species which have thus far been analyzed, human milk contains less proteid and ash than the milk of any other species.

While, in the present state of our knowledge, but little is known of the actual forms of combination in which the several mineral constituents of milk exist in the milk itself,<sup>10</sup> it seems reasonable to conceive that only those basic elements of the milk which exist therein in organic combination, *viz.*, in combination with organic acids and proteids, and which on oxidation are primarily convertible into carbonates, would ultimately become available in the processes of metabolism for the neutralization of acids produced within the organism, and hence able ultimately to safeguard the organism against the necessity for the production and withdrawal of ammonia and the train of disturbances met with in acidosis. These, together with the chlorides and a portion of the phosphates, compose the portion of the mineral matter actually essential to the processes of normal metabolism. It seemed of interest, therefore, to determine the amount of alkali in the ash of human and cow's milk.<sup>11</sup> Accordingly such determinations have been made on a number of specimens of cow's milk and human milk in the following manner:<sup>12</sup> A known quantity of the milk, approximately 5 gm., was evaporated to dryness on the

<sup>10</sup> See SOLDNER: *Landwirtschaftlichen Versuchs-Stationen*, 1888, xxxv, pp. 351-436.

<sup>11</sup> It has long been known that the ash of vegetable tissues and plant reserve substances is, as a rule, strongly alkaline, whereas the ash of animal tissues and animal reserve substances may be either alkaline or acid. Excellent instances of this are furnished by spinach and by the white and yolk of egg. According to my own determinations, 1 gm. of spinach (undried plant) yields, on incineration 0.02622 gm. of ash, having an alkalinity toward phenolphthalein equivalent to 2.61 c.c. of tenth-normal sodium hydroxide. The ash of the white of egg is also alkaline as may be seen from Table VI of this communication. On the other hand, the ash of the yolk of egg is acid toward phenolphthalein; the ash from 1 gm. of the undried yolk requires 0.1127 c.c. of tenth-normal sodium hydroxide to neutralize it. During recent years considerable attention has been paid to the reaction of the ash of vegetable and animal products, particularly to the degree of alkalinity of the ash of fruits and fruit products, by food chemists, with the view of detecting adulterations, and still more recently, in consequence of the great interest in all questions relating to the general subject of acidosis, the subject of the balance of the acid-forming and base-forming elements in foods has engaged the attention of certain physiological chemists, among them SHERMAN and SINCLAIR (see *Journal of biological chemistry*, 1907, iii, pp. 307-309).

<sup>12</sup> Through the kindness of Dr. A. M. PETER, Chemist of the Kentucky Agricultural Experiment Station, Lexington, Ky., a number of determinations of the alkalinity of the ash of the mixed milk of a herd of high-grade Jersey cows at the station were made for me for the sake of comparison. The results of Dr. PETER's determinations are given in Table III.

TABLE I.  
HUMAN MILK.

No. of sample.	Amount of milk taken. Grams.	Weight of ash. Grams.	Per cent of ash.	N/10 acid added. c.c.	N/10 NaOH required. c.c.	Alkalinity of milk in c.c. of N/10 NaOH.	Alkalinity per gm. of milk in c.c. of N/10 NaOH
				(H <sub>2</sub> SO <sub>4</sub> )			
1	4.9900	0.0130	0.261	5	4.20	0.80	0.1603
2	4.9723	0.0133	0.267	5	4.15	0.85	0.1709
3	5.0135	0.0179	0.356	5	3.70	1.30	0.2593
4	4.9902	0.0186	0.374	5	3.85	1.15	0.2304
5	5.0109	0.0169	0.337	5	3.70	1.30	0.2594
6	4.9755	0.0200	0.402	5	3.90	1.10	0.2218
7	4.9755	0.0136	0.273	5	4.15	0.85	0.1708 <sup>1</sup>
8	4.9656	0.0130	0.213	5	4.25	0.75	0.1514
9	5.0050	0.0130	0.259	5	4.20	0.80	0.1598
10	5.0027	0.0130	0.259	5	4.10	0.90	0.1799
11	4.9905	0.0199	0.398	5	4.00	1.00	0.2003
12	4.9457	0.0206	0.438	5	3.90	1.10	0.2224
13	5.9892	....	....	5	4.00	1.00	0.1669
14	5.0100	0.0137	0.273	5	4.25	0.75	0.1497
15	5.0310	0.0114	0.226	5	4.30	0.70	0.1391
16	2.6320	....	....	5	4.55	0.45	0.1709
17	5.0003	0.0112	0.223	5	4.35	0.65	0.1299
18	5.0969	....	....	5	4.25	0.75	0.1471
19	5.0603	....	....	5	4.15	0.85	0.1679
20	5.0316	....	....	5	4.25	0.75	0.1490
21	5.0427	0.0104	0.206	5	4.30	0.70	0.1388
<sup>1</sup> Specimen No. 7 was colostrum.							

TABLE I (continued).

No. of sample.	Amount of milk taken. Grams.	Weight of ash. Grams.	Per cent of ash.	N/10 acid added. c.c.	N/10 NaOH required. c.c.	Alkalinity of milk in c.c. of N/10 NaOH.	Alkalinity per gm. of milk in c.c. of N/10 NaOH.
22	5.0139	0.0154	0.307	(H <sub>2</sub> SO <sub>4</sub> ) 5	3.85	1.15	0.2293
23	4.9534	0.0157	0.317	5	4.00	1.00	0.2018
24	4.9803	0.0138	0.277	5	4.20	0.80	0.1602
25	5.0160	0.0133	0.265	(HCl) 5	4.40	0.60	0.1196
26	5.0305	0.0134	0.266	5	4.30	0.70	0.1391
<p>Specimens Nos. 1 to 25 inclusive were obtained from ten different women, the specimens from each woman being grouped together in the above table.</p> <p>Specimen No. 26 was a mixed sample consisting of the milk from four women.</p> <p>In specimens Nos. 25 and 26, tenth-normal hydrochloric acid was used in making the alkalinity determination.</p>							

steam bath and incinerated at low red heat until all of the carbon had been consumed. The ash was then weighed, and 5 c.c. of tenth-normal sulphuric or hydrochloric acid added, together with a small amount of water. The mixture of ash and acid was then gently warmed in order to remove any carbon dioxide which might possibly result from the decomposition of carbonates, allowed to cool, and titrated with tenth-normal sodium hydroxide, using phenolphthalein as an indicator. The results of these determinations are given in Tables I and II.

It will be seen from these results that the ash of human milk and that of cow's milk exhibit the same degree of alkalinity towards phenolphthalein as an indicator.<sup>18</sup>

<sup>18</sup> In all investigations of this kind the choice of an indicator and of a method for determining the alkalinity is obviously a matter of considerable importance. In this connection it has been found that if the ash of human and cow's milk be boiled with water the solution thus obtained is alkaline towards phenolphthalein. The ash obtained from 5 gm of human milk and that from 5 gm. of cow's milk were found to require approximately the same amount of tenth-normal hydrochloric acid, *vis.*, 0.2 c.c. for the ash of human milk and 0.15 c.c. for the ash of cow's milk. On standing after being thus neutralized, the solutions tend to become alkaline

TABLE II.

Cow's MILK.

No. of sample.	Amount of milk taken. Grams.	Weight of ash. Grams.	Per cent of ash.	N/10 acid added. c.c.	N/10 NaOH required. c.c.	Alkalinity of milk in c.c. of N/10 NaOH.	Alkalinity per gm. of milk in c.c. of N/10 NaOH.
				(H <sub>2</sub> SO <sub>4</sub> )			
1	5.0286	0.0369	0.734	5	4.15	0.85	0.1690
2	4.9350	0.0317	0.642	5	4.20	0.80	0.1620
3	4.9934	0.0338	0.676	5	4.00	1.00	0.2002
4	5.0178	0.0339	0.675	5	4.25	0.75	0.1494
5	5.0282	0.0378	0.751	5	4.20	0.80	0.1591
6	5.0423	0.0364	0.721	5	4.20	0.80	0.1586
7	5.0164	0.0371	0.739	5	4.20	0.80	0.1594
8	5.0193	0.0368	0.733	5	4.25	0.75	0.1494
9	5.0204	0.0365	0.727	5	4.15	0.85	0.1695
10	5.0167	0.0383	0.763	10	9.25	0.75	0.1491
11	5.0188	0.0363	0.723	5	4.15	0.85	0.1693
12	5.0070	0.0369	0.736	5	4.10	0.90	0.1797
13	5.0480	0.0359	0.711	5	4.00	1.00	0.1980
14	4.9644	0.0327	0.658	5	4.25	0.75	0.1510
15	5.0319	0.0370	0.735	5	4.10	0.90	0.1788
16	5.0150	0.0357	0.711	5	4.00	1.00	0.1994
17	5.0165	0.0358	0.714	5	3.75	1.25	0.2491
18	5.0117	0.0329	0.656	5	4.30	0.70	0.1396
19	5.0564	0.0378	0.747	5	3.45	1.55	0.3065
20	5.0528	0.0389	0.769	5	3.40	1.60	0.3162
21	5.0261	0.0361	0.718	5	4.25	0.75	0.1492
22	5.0324	0.0371	0.737	5	4.10	0.90	0.1788
23	5.0369	0.0359	0.710	5	4.30	0.70	0.1389
24	5.0351	0.0365	0.724	5	4.20	0.80	0.1588
25	5.0455	0.0363	0.719	5	3.85	1.15	0.2011
26	5.0260	0.0363	0.722	5	4.15	0.85	0.1691
27	5.0286	0.0349	0.694	5	4.25	0.75	0.1491

# Alkali in the Ash of Human and Cow's Milk. 291

TABLE II (continued).

No. of sample.	Amount of milk taken. Grams.	Weight of ash. Grams.	Per cent of ash.	N/10 acid added. c.c.	N/10 NaOH required. c.c.	Alkalinity of milk in c.c. of N/10 NaOH.	Alkalinity per gm. of milk in c.c. of N/10 NaOH.
28	5.0305	0.0386	0.767	H <sub>2</sub> SO <sub>4</sub> 5	3.85	1.15	0.2286
29	5.0603	0.0386	0.762	5	3.90	1.10	0.2173
30	5.0417	0.0345	0.684	5	4.20	0.80	0.1586
31	5.0441	0.0342	0.678	5	4.25	0.75	0.1486
32	5.0474	0.0370	0.733	(HCl) 5	4.25	0.75	0.1485
33	5.0383	0.0356	0.708	5	4.10	0.90	0.1789
34	5.0188	0.0350	0.697	5	4.30	0.70	0.1391
35	5.0271	0.0363	0.722	5	4.40	0.60	0.1193
36	5.0280	0.0397	0.789	5	3.80	1.20	0.2386
37	5.0303	0.0363	0.721	5	4.10	0.90	0.1789
38	5.0127	0.0373	0.744	5	3.80	1.20	0.2393
<p>Milks Nos. 1 to 20 inclusive and Nos. 34 to 38 inclusive were obtained direct from a herd supplying milk to the local market.</p> <p>Milks Nos. 21 to 27 inclusive were from normal cows belonging to the herd of the Experiment Station of the Bureau of Animal Industry, Department of Agriculture, Bethesda, Md.</p> <p>Milks Nos. 28 to 31 inclusive were from tuberculous cows of the same herd as Nos. 21 to 27.</p> <p>Milks Nos. 32 and 33 were mixed samples from a large local dairy.</p> <p>In samples Nos. 32 to 38 inclusive, tenth-normal hydrochloric acid was used in making the alkalinity determinations.</p>							

again, that of the human milk somewhat more rapidly and strongly than that of the cow's milk. It therefore occurred to me to employ phenolphthalein as the indicator for these determinations, and to determine the alkalinity by the method indicated in the above, for the reason that the chemical reactions involved in the determination of the alkalinity of the ash when this indicator is employed seem to approximate most closely the conditions actually met with in the body so far as the absorption and elimination of the phosphates are concerned. Recently FARNSTEINER (*Zeitschrift Nahr.-Genussm.*, xiii, pp. 305-338) has proposed a somewhat more complicated method for determining the alkalinity of the ash of various foods, involving the use of neutral calcium chloride and ammonium chloride for the purpose of removing all of the phosphates by precipitation in the presence

TABLE III.

COW'S MILK. MIXED SAMPLES FROM THE HERD AT THE AGRICULTURAL EXPERIMENT STATION, LEXINGTON, KY.

(DETERMINATIONS MADE BY DR. A. M. PETER.)									
Date.	Sp. gr. by hydrometer.	Temperature in degrees C.	Per cent of fat Babcock.	Total solids for mean fat, corrected sp. gr. per cent.	Total solids by evaporation by wt. per cent.	Weight of 5 c.c. taken for evap. for solids and ash.	Per cent of ash by weight.	Alkalinity in c.c. N/10 KOH.	Alkalinity per gm. in c.c. of N/10 KOH.
May 11, 1	1.031	22	5.2	14.53	14.84	5.0259	0.780	0.90	0.1797
" 2	....	..	5.4		14.86	5.0180	0.779	0.80	0.1594
May 12, 1	1.031	23	5.2	14.70	14.74	5.0497	0.778	0.80	0.1584
" 2	....	..	5.6		14.74	5.0550	0.773	0.80	0.1582
May 13, 1	1.032	22	5.4	15.06	14.80	5.0725	0.761	1.35	0.2661
" 2	....	..	5.5		14.80	5.0447	0.767	1.35	0.2684
May 14, 1	1.031	22½	5.3	14.68	14.83	5.0470	0.765	1.25	0.2476
" 2	....	..	5.4		14.84	5.0515	0.762	1.20	0.2372
May 15, 1	1.031	22	5.4	14.65	14.73	5.0406	0.757	0.85	0.1686
" 2	....	..	5.4		14.75	5.0411	0.773	0.90	0.1785
May 16, 1	1.032	22½	5.3	14.83	14.81	5.0684	0.740	0.75	0.1479
" 2	....	..	5.2		14.76	5.0785	0.746	0.75	0.1479
Mean . .			5.4	14.74	14.80		0.765		0.1931
<p>In the determinations of May 11 and 12, no crystallization of <math>\text{CaSO}_4</math> was observed.</p> <p>The milks of May 13 and 14 were left on the water-bath longer than was intended, and some <math>\text{CaSO}_4</math> crystallized out.</p> <p>In the determinations of May 15 there was a slight separation of <math>\text{CaSO}_4</math>.</p> <p>Determinations of May 16 were diluted with sufficient water to prevent the separation of <math>\text{CaSO}_4</math>.</p>									

of an excess of standard ammonia, the titration being made on the clear solution after the precipitation of the phosphates, with standard hydrochloric acid, using methyl orange as an indicator. This author points out that on account of the high content of lime and magnesia in milk, the difference in alkalinity by his method and that of direct titration with phenolphthalein is not great in spite of the large amount of phosphoric acid which milk contains. This has been my own experi-

It should be borne in mind that when a solution of the ash of milk in acid is neutralized with caustic soda, a precipitate of triphosphates is formed, and if the composition of the ash is such that there is an excess of phosphoric acid over and above that required to form the triphosphates of iron, calcium, and magnesium, the solution will become neutral and show the end-point of the titration towards phenolphthalein as an indicator when a quantity of the alkali has been added in sufficient amount to convert the excess of phosphoric acid into diphosphates of the alkali metals. Such being the case, it seemed of interest in this connection to calculate the alkalinity of the ash of cow's milk and human milk from the results of complete ences. Thus with FARNSTEINER's method I obtained the following results with human and cow's milk :

Sample.	Amount of milk taken. Grams.	Weight of ash. Grams.	Per cent of ash.	N/10 HCl required. c.c.	N/10 NH <sub>4</sub> OH added. c.c.	Alkalinity equivalent to N/10 HCl. c.c.	Alkalinity of 1 gm. of milk in c.c. of N/10 NaOH.
Cow's milk <sup>1</sup>	5.1340	0.0381	0.742	11	10	1	0.1948
Human milk <sup>2</sup>	5.0497	0.0109	0.215	11	10	1	0.1983
<sup>1</sup> This sample of cow's milk showed an alkalinity of 0.1753 c.c. of tenth-normal caustic soda per gram, by direct titration, using phenolphthalein as the indicator. <sup>2</sup> This sample of human milk showed an alkalinity of 0.1386 c.c. of tenth-normal caustic soda per gram, by direct titration, using phenolphthalein as the indicator.							

It will be seen that the two methods give reasonably concordant results, and that according to both methods the ash of human and that of cow's milk show approximately the same degree of alkalinity. On the other hand, when the ash of human and cow's milk is dissolved in an excess of tenth-normal hydrochloric acid and titrated with tenth-normal sodium hydroxide, using dimethyl-amido-azo-benzol as the indicator, much higher values for the alkalinity of the ash of the two kinds of milk are obtained, especially for the ash of cow's milk, as may be seen from the determinations given in the table on p. 294.

(The results of the alkalinity determinations on these specimens of human and cow's milk in which phenolphthalein was used as the indicator are given in Tables I and II, under the same serial numbers.)

It will be seen, therefore, that when dimethyl-amido-azo-benzol is employed as the indicator, the alkalinity of the ash of the two milks is approximately proportional to the amounts of ash in the two kinds of milk. These higher alkalinity values are due to the relatively large amounts of phosphates which the two milks

analyses of the ash of these milks, on the assumption that, in the titration as carried out in our experiments, the iron, magnesium, and calcium would form triphosphates so far as the several amounts of these bases and the phosphoric acid present in the ash would per-

## COW'S MILK.

Sample.	Amount of milk taken. Grams.	Weight of ash. Grams.	Per cent of ash.	N/10 HCl added. c.c.	N/10 NaOH required. c.c.	Alkalinity of milk in c.c. of N/10 NaOH.	Alkalinity per gram of milk in c.c. of N/10 NaOH.
32	5.0474	0.0370	0.733	5	1.70	3.30	0.6538
33	5.0383	0.0356	0.708	5	1.60	3.40	0.6760
34	5.0188	0.0350	0.697	5	1.50	3.50	0.6973
35	5.0271	0.0363	0.722	5	1.65	3.35	0.6663
36	5.0280	0.0397	0.789	5	1.10	3.90	0.7756
37	5.0305	0.0363	0.721	5	1.20	3.80	0.7554
38	5.0127	0.0373	0.744	5	1.05	3.95	0.7880
Mean	.....	....	....	..	...	...	0.7160
HUMAN MILK							
25	5.0160	0.0133	0.265	5	3.75	1.25	0.2492
26	5.0305	0.0134	0.266	5	3.80	1.20	0.2385
Mean	....	....	....	..	...	...	0.2438

contain, and the difference in alkalinity to the difference in the amounts of phosphates in the two kinds of milk. In this connection it should be borne in mind that the end point with this indicator is reached when the phosphoric acid present in the acid solution of the ash has been converted into monophosphates, and that under these circumstances none of the earths are precipitated out of the solution as triphosphates. On the other hand, it is very unlikely that all of the phosphates of the food in their gradual passage through the body are ever completely in the form of monophosphates, except possibly in the stomach, so that, as indicated by dimethyl-amido-azo-benzol, the ash of human and that of cow's milk, especially the latter, neutralize larger amounts of acid than they could ever neutralize under conditions normally prevailing in the organism. It is believed that these conditions are more approximately realized when phenolphthalein is used as the indicator, or when, as in FARNSTEINER'S method, the phosphates are removed altogether, and it was for this reason that phenolphthalein was used as the indicator in this work.



mit, any excess of phosphoric acid going to form a diphosphate of sodium or potassium. In this calculation the results of the analyses of the ash of human and cow's milk given in Albu and Neuberg's "Mineralstoffwechsel," page 52, have been employed. The results of these calculations are given in Tables IV and V.

TABLE IV.

**Cow's MILK.**

[illegible]

It will be seen that the calculated values of the alkalinity of human and cow's milk are, like the observed values, approximately equal. It will also be seen that the observed values are slightly higher than the values calculated from the results of the analyses of the ash of the two kinds of milk. These discrepancies are easily explained. In the determination of the alkalinity of the ash it has been found

that in some instances small amounts of calcium sulphate separated as the result of heating the solution of the ash in sulphuric acid. In

TABLE V.

HUMAN MILK.

CONSTITUENTS.							
Combined with <sup>1</sup>	Per cent of						
	P <sub>2</sub> O <sub>5</sub> .	Cl.	Fe <sub>2</sub> O <sub>3</sub> .	MgO.	CaO.	K <sub>2</sub> O.	Na <sub>2</sub> O.
P <sub>2</sub> O <sub>5</sub> . . . . .	....	....	0.06	...	15.91		
C <sup>1</sup> . . . . .	....	....	...	2.60	0.49	19.54	
Fe <sub>2</sub> O <sub>3</sub> . . . . .	0.053						
MgO . . . . .	....	4.62					
CaO . . . . .	13.447	0.62					
K <sub>2</sub> O . . . . .	....	14.76					
Na <sub>2</sub> O . . . . .							
Total combined	13.50	20.00	0.06	2.60	16.40	19.54	
Free . . . . .	....	....	...	...	....	11.86 <sup>2</sup>	11.90
Total . . . . .	13.50	20.00	0.06	2.60	16.40	31.40	11.90

<sup>1</sup> The figures in the above tables (IV and V) show the amounts of the constituents at the heads of the vertical columns combined with the constituent in the same horizontal line. For example, of the total of 13.50 per cent of P<sub>2</sub>O<sub>5</sub> in Table V above, 0.053 per cent is combined with Fe<sub>2</sub>O<sub>3</sub> and 13.447 per cent is combined with CaO.

<sup>2</sup> Free K<sub>2</sub>O calculated as Na<sub>2</sub>O . . . = 7.81 per cent.  
 Free Na<sub>2</sub>O . . . . . = 11.90 "  
 Total alkalinity of the ash in terms  
 of per cent of Na<sub>2</sub>O . . . . . = 19.71 "  
 Per cent of ash in human milk . . = 0.20 "  
 1 gm. of human milk contains . . = 0.002 gm. of ash.  
 Available alkali in ash of 1 gm. . . = 0.0003942 gm. Na<sub>2</sub>O.  
 = 0.0005086 gm. NaOH.  
 = 0.1271 c.c. N/10 NaOH solution.

these determinations, therefore, an equivalent quantity of phosphoric acid was neutralized by two equivalents of caustic soda, whereas, if the calcium precipitated as sulphate had remained in

solution, this phosphoric acid would have been precipitated as tri-calcium phosphate, more tenth-normal alkali would have been required to effect the neutralization, and therefore the alkalinity of the ash would have been correspondingly less. Therefore in those instances in which calcium sulphate was precipitated, the ash of the milk appeared to be more alkaline than it really was.<sup>14</sup> Furthermore, in calculating the degree of alkalinity of the ash of human and cow's milk from the recorded analyses of the milk by other chemists, the calculation has been based upon a total percentage of 0.2 per cent for human milk and 0.7 per cent for cow's milk, whereas in the milks with which we worked the actual amounts of ash found were, as a rule, higher than these figures, our human milks averaging 0.294 per cent ash, and our cow's milks 0.72 per cent, while the cow's milks analyzed by Dr. Peter averaged 0.765 per cent of ash. Obviously, therefore, the found values for these milks with high percentages of ash will be correspondingly higher than the calculated values. It will be seen, further, that in 35 out of the 50 specimens of cow's milk in which the alkalinity of the ash was determined, it ranged from 0.1193 c.c. of tenth-normal sodium hydroxide to 0.1788 c.c. the average being 0.1574 c.c., and that in 18 out of 26 specimens of human milk in which the alkalinity of the ash was determined, it ranged from 0.1196 to 0.1799 c.c. of tenth-normal sodium hydroxide, the average being 0.1513 c.c., which numbers agree reasonably well with the average alkalinity of the ash of the two kinds of milk calculated from the complete analyses.

It is evident, therefore, that while cow's milk contains from 2.5 to 3.5 times as much mineral matter as human milk, the ash of the two milks contains approximately the same amount of available alkali. If, for example, the degree of alkalinity of the ash of human milk be arbitrarily made equal to unity, then the alkalinity of the ash of cow's milk calculated from the complete analyses of other observers is 1.04, or calculated from the results of the alkalinity determinations given in Tables I, II, and III, it is 1.01. It would

<sup>14</sup> Dr. PETER also arrived at the conclusion, quite independently of my findings, that some of his figures were too high in consequence of the separation of calcium sulphate, and he is also of the opinion that the true alkalinity value of 5 gm. of the milk examined by him is not far from 0.75 c.c. of tenth-normal potassium hydroxide, which would correspond to 0.15 c.c. of tenth-normal sodium hydroxide per gram of milk, which number agrees quite closely with the degree of alkalinity of the ash of 1 gm. of cow's milk calculated from the results of the complete analyses of the ash of cow's milk.

seem, therefore, that while cow's milk contains a much larger amount of mineral matter than human milk, it can supply the organism of the infant with only about the same amount of available alkali as that contained in human milk. In this connection it is interesting to note that Heubner<sup>15</sup> and his co-workers, and also Blauberg<sup>16</sup> from extensive experimental studies on the mineral metabolism of breast-fed and artificially fed infants, have found that while in the nutrition of the infant 80 per cent of the ash of human milk is absorbed only 60 per cent of the ash of cow's milk is absorbed. It would seem, therefore, that the salient points of difference between the two kinds of milk are: (1) human milk contains relatively more of its mineral matter in utilizable form than cow's milk; (2) it can supply the organism of the child with relatively larger amounts of available alkali in proportion to the proteid than cow's milk; (3) it contains much less proteid; and (4) it contains a more readily absorbable variety of fat.

It is believed that these differences in composition between the two kinds of milk are of interest as throwing light upon certain phases of infant nutrition which in the present state of our knowledge are more or less difficult to understand. That such is the case seems evident from the following considerations: As is well known, the greater number of the older attempts at artificial infant feeding had for their object the selection and preparation of an infant food which would approximate as closely as possible the composition of human milk. Generally this was accomplished by the dilution of cow's milk and the addition thereto of cream, milk sugar, white of egg, alkaline citrates, lime water, etc., and various claims have been made by different pediatricists and others regarding the good results obtained by these several methods, into the merits of which it is needless to enter at any great length in this connection. Suffice it to say, that apparently many of them left much to be desired so far as meeting the food requirements of the infant is concerned, and the maintenance of its physical well-being. On the other hand, during recent years it has been established, chiefly through the labors of Czerny and Keller<sup>17</sup> in Germany, and Budin<sup>18</sup> in Paris, and confirmed by Bren-

<sup>15</sup> HEUBNER: *Deutsche Aerzte-Zeitung*, 1901, iii, pp. 481-483.

<sup>16</sup> BLAUBERG: *Zeitschrift für Biologie*, 1900, xl, pp. 1-53.

<sup>17</sup> CZERNY and KELLER: *Des Kindes Ernährung, Ernährungsstörungen und Ernährungstherapie*, 2d Abt., Leipzig u. Wien, 1901.

<sup>18</sup> BUDIN: *The nursling*, English translation by Maloney, London, 1907.

nemann<sup>19</sup> and Walls<sup>20</sup> in this country, that the proteids of cow's milk are practically as easily digestible by the suckling as those of human milk, and that the digestive disturbances in infants which were formerly ascribed to the excess and indigestibility of cow's milk proteid are in reality due to an excess of fat. According to these authors, the immediate cause of the conditions met with in atrophic and marantic children, and those which result from over-feeding with cow's milk, is, in most instances at least, primarily an acidosis, as shown by the appearance of ammonia in the urine, and by the characteristic dry, hard, pale feces, which have been found to consist largely of the insoluble salts of the fatty acids (*Seifenstuhlen*). These authors have shown that an excess of fat in the food of infants results in the withdrawal of alkalis from the tissues by the fatty acids produced in the intestines, so that ammonia is ultimately drawn upon to neutralize the normal acid products of metabolism. While no one can question the excellent results which have been obtained by these and other observers in the artificial feeding of infants upon whole sterilized cow's milk, not too rich in fat, and while no one could question the general correctness of these ideas, namely, that the primary cause of the digestive disturbances in infants fed upon cow's milk containing large amounts of fat is the difficulty of fat absorption in the intestinal tract of the child, and that the immediate cause of these gastro-intestinal disturbances is an acidosis growing out of the withdrawal of alkaline substances required in the normal metabolism of the organism, it must of necessity impress one as remarkable that the two kinds of milk, containing, as they do, approximately the same amount of fat, should nevertheless conduct themselves so differently in the nutrition of the child. It is believed, therefore, that while the primary cause of the gastro-intestinal disturbances following the use of cow's milk in infant feeding is due to the fact that the fat is not readily absorbed, the more remote and fundamental cause of these disorders in infants fed upon rich cow's milk is an excessive proteid metabolism and an insufficiency in available mineral matter in cow's milk as compared with human milk. It has long been recognized that a certain amount of proteid is needed each day for the processes of growth and also to make good

<sup>19</sup> BRENNEMANN: *Journal American Medical Association*, 1907, xlviii, pp. 1338-1344.

<sup>20</sup> WALLS: *Ibid.*, pp. 1389-1392.

the loss of tissue broken down in endogenous or tissue metabolism. From the admirable researches of Folin<sup>21</sup> and of Chittenden<sup>22</sup> in this field it is now coming to be recognized, however, that the quantities of proteid supplied by ordinary and standard diets are generally, at least so far as the full-grown man is concerned, considerably in excess of the requirements of the organism so far as the maintenance of the nitrogen balance and the necessary proteid reserve are concerned. In other words, according to these authors, the exogenous proteid metabolism is usually excessive, and greatly above the ordinary requirements of the body. While it must be admitted, of course, that the rapidly growing infant probably requires more proteid for the purposes of growth and tissue development than would be required for a full-grown man to maintain himself in nitrogenous equilibrium on a mixed diet, it must be admitted, from the results gained by practical experience, that the mother's milk, in the quantity usually supplied the infant, is amply sufficient to meet the requirements of the infant organism both for proteid and energy, and that in the quantity ordinarily supplied the amount of proteid in human milk is, weight for weight, greatly in excess of that demanded by a full-grown man upon a low proteid diet. That such is the case is evident from the following: According to de Mattos<sup>23</sup> a healthy breast-fed infant, weighing the first week 3350 gm. and at the end of thirty weeks 8226 gm., consumed per day a quantity of human milk ranging from 264 to 1030 gm., or an average of 895.2 gm. of milk per day. The mean weight of this infant during the period of observation was 5788 gm. On the assumption that the human milk furnished the child contained 1.5 per cent of proteid, the child was metabolizing 13.43 gm. of proteid per day, or 2.32 gm. of proteid per kilogram of body weight. For a man weighing 70 kg., therefore, such a diet would mean the consumption of 10.8 liters of human milk per day, and an intake of 162.4 gm. of proteid. On the basis of Chittenden's calculations this is nearly three times the amount actually required to maintain a healthy man in nitrogenous equilibrium and meet the nitrogenous food requirements of his organism.

Judged by the requirements for a man, therefore, this would be an excessive proteid diet. Upon teleological grounds, however, and

<sup>21</sup> FOLIN: This journal, 1905, xiii, p. 107.

<sup>22</sup> CHITTENDEN: The nutrition of man, New York, 1907.

<sup>23</sup> DE MATTOS: Jahrbuch für Kinderheilkunde und physikalische Erziehung, 1902, lv, p. 47.

so far as we may judge from the results of practical experience with breast-fed infants, this diet is probably not excessive for the growing infant, or at least not greatly so, the excess of proteid over and above that required for the same body weight of the full-grown man being utilized for purposes of growth, which in the case of this particular infant amounted in round numbers to 5 kg. in thirty weeks. On the other hand, this same infant, if fed by Budin's system upon sterilized whole cow's milk containing 3.75 per cent of fat, would probably have received per day an amount of cow's milk equal in weight to the amount of human milk consumed, *viz.*, 895.2 gm., or perhaps even more, since, according to this distinguished pediatricist, the normal growing infant during the first few weeks of its life must be given a little over one fifth of its body weight of cow's milk per day, and later a gradually diminishing amount of milk depending upon the age, growth, and general condition of the particular infant. Had the infant under investigation by de Mattos received the same amount of cow's milk as breast milk daily during the period of observation, it would have metabolized daily, in round numbers, 26 gm. of proteid, or 4.5 gm. of proteid daily per kilogram of body weight. For a man weighing 70 kg. such a diet would amount to a consumption of 10.8 liters of cow's milk per day and to a proteid intake of at least 315 gm., or at least five times as much as that needed to meet his daily requirements. Certainly this quantity of proteid would, in the light of our present knowledge, be regarded as excessive for the average man. That it is also excessive for the child, or at least considerably more than is needed for normal growth, is indicated by the fact that upon a diet consisting of the same amount of human milk and containing only half this amount of proteid, he was able to grow and thrive actively. To take another case by way of illustration: The infant son of Dr. Goldberger of this laboratory, now 11 months old and weighing 9639 gm. (21¼ lbs.), is at present receiving daily 1105.6 gm. (39 oz.) of sterilized whole cow's milk (high-grade market milk), containing 3.25 per cent of fat, diluted with a small amount of barley water. In addition to this he receives the juice of one orange daily. The daily diet of this infant, therefore, would, for a man weighing 154 lbs. (70 kg.), amount to a total intake, in round numbers, of 8 liters of milk per day, and on the supposition that the milk contains 3 per cent of proteid, to a total intake of proteid amounting to 240.4 gm. per day. There can be no doubt, therefore, that as a rule artificially fed children on a diet of

whole cow's milk are really being nourished upon a high proteid diet. As pointed out by Chittenden, however, the bad results following a high proteid diet are, in the case of man, only slowly cumulative, and probably more often than not manifest themselves only after years of such living. The disorders usually attributed to a high proteid diet, such as gout, rheumatism, etc., are characteristic of age rather than childhood, and so far as we are able to judge from the large practical experience of such observers as Budin, sterilized whole cow's milk agrees perfectly with the normal infant; indeed it has been used to the greatest possible advantage in the nourishment of the weakling, provided that the percentage of fat in the milk be not allowed to exceed 3.75 per cent (Budin), or the quantity of proteid present in the milk (Walls), and according to the statement of Dr. Goldberger, the father of the infant referred to above, his baby seems to be able to assimilate almost any amount of cow's milk proteid, provided that the fat of the milk is not allowed to exceed 3.25 per cent as a maximum. On the other hand, Hunt<sup>24</sup> has shown that diets rich in certain proteids undoubtedly render an animal more susceptible to the action of certain poisons, such as acetonitrile, and doubtless many cases of acute digestive disturbances in artificially fed children are due either directly or indirectly to excessive amounts of proteid.

It would seem, further, that the acidosis ultimately resulting from the feeding of cow's milk too rich in fat and which according to most authors is primarily attributable to lack of absorption and assimilation of the fat and to the withdrawal of excessive amounts of mineral matter from the organism of the child, is in reality the result of an excessive proteid metabolism, resulting from the nourishment of the infant on a food stuff rich in proteid (cow's milk), and which compared with the normal diet of the child (human milk) is relatively poorer in utilizable mineral matter and available alkali.

While as yet very little is known as to the precise mode of action of the several mineral substances contained in living cells and tissues, and always found in intimate association with the natural proteids, their importance to metabolism and to the normal activity of living protoplasm wherever this is met with can scarcely be overestimated, and is so well understood as scarcely to call for comment in this connection. That such is the case, however, is indicated by the uni-

<sup>24</sup> HUNT: Proceedings of the Society for Experimental Biology and Medicine, 1906, iii, p. 16.



versal occurrence of various mineral substances in all living cells of the plant and animal, and by their active participation in all vital phenomena with which we are familiar, such as the growth and formation of new tissue, the contraction of heart muscle, the irritability of muscle and nerve generally, the production of active glandular secretions, the storing up and utilization of reserve materials both in the animal and plant, the fertilization of the ovum, its segmentation, and the nourishment of the embryo. According to Albu and Neuberg, mineral substances probably play an important rôle in the so-called processes of intermediary metabolism, especially in the glandular tissues, and are largely concerned in the decomposition and assimilation of organic substances. As is well known, they govern the osmotic pressure within the cell and tissues and in the blood and juices of the organism. They regulate the reaction of the blood and tissue juices, as well as the course of many fermentative processes, especially such as occur in the alimentary canal.<sup>25</sup>

In this connection an interesting relationship has been recognized by von Bunge<sup>26</sup> as existing between the several amounts of proteid and ash in the milk of a number of different animals. According to this author the remarkable differences in the composition of the milk of different animals find their simplest explanation in the corresponding differences in the rate of growth of the sucklings of the several species, as may be seen from the following table:

Animal.	Parts per 100		Time required to double the body weight of the new-born animal in days.
	Proteid.	Ash.	
Man . . . . .	1.4	0.22	180
Horse . . . . .	1.8	0.41	60
Cow . . . . .	4.0	0.80	47
Dog . . . . .	9.9	1.31	9

<sup>25</sup> For a more exhaustive discussion of the part played by the mineral constituents of protoplasm in life processes, see ALBU and NEUBERG: *Physiologie und Pathologie des Mineralstoffwechsels*, Berlin, pp. 108 *et seq.*

<sup>26</sup> VON BUNGE: *Die zunehmende Unfähigkeit der Frauen ihre Kinder zu stillen*, München, 1905.

From this he arrives at the conclusion that the more rapidly the suckling grows, the greater the needs of the organism for those food stuffs which serve for the building up of the tissues, *viz.*, proteids and salts. Forster's experiments on dogs with a diet composed of ash-free carbohydrates and fats and meats containing only small amounts of salts point to the same conclusion, while Lunin's experiments on mice fed with dried milk and with the ash-free constituents of milk would seem to indicate that some of the salts must be present in the form of organic combination such as are ordinarily met with in animal and vegetable foods.<sup>27</sup> Such salts would, of course, yield a corresponding amount of available alkali on incineration and would obviously go to increase the alkalinity of the milk ash. That the ash constituents of various food stuffs are essential to the maintenance of a normal condition of health in man is indicated by the train of disturbances which have followed all attempts on the part of various observers to subsist for any length of time on an ash-free diet.<sup>28</sup> The part played by alkalis or by salts exhibiting a faintly alkaline reaction in the intestinal digestion and absorption of proteids and fats and also in favoring the cleavage of proteids and fats by the intracellular proteolytic and lipolytic ferments respectively, to say nothing of the fact that the oxidation of carbohydrates and various other complex organic compounds is greatly accelerated by the presence of alkali, is at present too well understood to require further comment, except as serving in this connection to direct attention to the important rôle played by the mineral substances of the body in the absorption and metabolism of food and the general bearing which such considerations may have on the ideas herein set forth. The fact that so close a proportionality exists between the percentage of the ash constituents of milk and the several amounts of proteids contained in the milk of different animals in itself indicates that the salts of milk are primarily concerned in proteid metabolism. In this connection Voit<sup>29</sup> regards it as absolutely proved that proteid fed to cells is the earliest of all food stuffs to be destroyed; next the carbohydrates, and lastly the fats. That it is readily absorbed in the intestine is indicated by the fact that practically none of the ingested

<sup>27</sup> Cited by HOWELL. See HOWELL's Text-book of physiology, Saunders, Philadelphia, 1906, p. 802.

<sup>28</sup> TAYLOR, A. E.: Studies on an ash-free diet, University of California publications, Pathology, 1904, i, p. 71.

<sup>29</sup> VOIT: Münchener medizinische Wochenschrift, 1902, xlix, p. 233, cited by LUSK, Nutrition of man, p. 42.

proteid is found in the feces. The result of this rapid absorption and metabolism of the large amounts of proteid supplied the infant when fed on cow's milk is a correspondingly rapid excretion of urea and salts by the kidneys. In this connection it should also be borne in mind that salts are necessary for the absorption and assimilation of fats. It is now generally believed that the neutral fats of foods are in the process of absorption completely split up by the pancreatic lipase into free fatty acids and glycerin. The former are rendered soluble by the bile and the sodium carbonate contained in the pancreatic juice. The resulting soap, free fatty acids, and glycerin are then absorbed by the epithelial cells of the intestines, and from these substances fat is synthesized in the lymph and in this manner rendered available for immediate nutrition or else stored up in the tissues as reserve material.

As the result of feeding the infant with cow's milk containing an amount of fat equal to that contained in human milk, the rapid metabolism of the relatively large amount of proteids contained in the cow's milk causes the rapid elimination of large amounts of salts through the kidneys, whereas, as the result of slow absorption of a somewhat difficultly absorbable fat in the gut, large quantities of mineral matter are withdrawn by way of the intestines and pass into the feces. This condition of affairs, coupled with the fact that by no means all of the available mineral matter contributed by the cow's milk itself is available to and utilizable by the organism, soon leads to an upsetting of the mineral balance, and the characteristic symptoms of acidosis and malnutrition supervene. When the child is fed on cow's milk, therefore, we have, with respect to the boundary lines presented by the intestinal mucosa, two opposing sources of mineral waste, — one accomplished by an excessive proteid metabolism within and draining out by way of the kidneys, and the other accomplished by delayed fat absorption and waste of mineral matter through the gut. Both deplete the cells of the body of available mineral matter, and so far as the actual removal of available mineral matter is concerned, either process may be regarded as supplemental to the other.<sup>80</sup>

<sup>80</sup> In this connection the high ash content of the feces of infants nourished on cow's milk as compared with the lower ash content of the feces of breast-fed infants is a matter of interest. Thus FORSTER found the dry feces of infants nourished on cow's milk to contain 34 per cent of ash, whereas WEGSCHEIDER found only 7.1 to 8.4 per cent of ash in the feces of breast-fed infants. ESCHERICH also noted

On the other hand, with a food stuff poorer in proteid and containing an equal amount of a more easily absorbable fat, and sufficient carbohydrate, in excess, to compensate for the excess proteid of the proteid-rich food, and which, while containing a much smaller amount of mineral matter, in reality contains as much available and utilizable mineral matter as the proteid-rich food, there is a sufficient amount of available mineral matter to accomplish the absorption and metabolism both of the proteid and of the fat, without drawing upon any of the mineral matter of the organism itself. Hence in the metabolism of such a food as human milk we have no fat acidosis and none of those gastro-intestinal disturbances which follow in the wake of cow's milk feeding, or, if so, these are merely the result of injudicious feeding of the infant by the mother, and may be easily corrected by greater care and discretion in nursing, or are due to some abnormality in the composition of the milk, such as an excess in the quantity of fat<sup>31</sup> or to some peculiar toxic or pathologic condition of the mother's milk, or to some idiosyncrasy on the part of the child.

It is believed that some of the most valuable recommendations and practices relating to infant feeding, such as the feeding of skimmed

the high ash content of the feces of infants fed on cow's milk, and more recently HEUBNER and his co-workers, and also BLAUBERG have found 6.9 and 6.6 per cent of ash respectively in the feces of breast-fed infants, and the former 20.7 to 29 per cent of ash in the feces of infants fed on cow's milk. Great emphasis has recently been laid upon these differences in the ash content of the feces of infants by HEUBNER (*Deutsche Aerzte-Zeitung*, 1901, pp. 481-483), as affording the simplest explanation of the observed differences in the color, consistency, and general characteristics of the feces of breast-fed and artificially nourished infants, and as throwing light on the rôle of the mineral constituents in the absorption and assimilation of food. According to this author, the transport of such large amounts of mineral matter through the organism as is involved in the absorption and assimilation of cow's milk cannot but greatly increase the work of the organism, and is a phase in the nutrition of the infant which in the future should not be allowed to go unheeded. The general opinion, therefore, of those who have given this subject the closest attention is that while human milk is poorer in mineral constituents than cow's milk, this deficiency is compensated for by a better utilization of the ash constituents of the human milk and by less waste of mineral matter through the urine and feces. (See ALBU and NEUBERG: *Loc. cit.*, p. 74.)

<sup>31</sup> In this connection it has been pointed out by BUDIN that even human milk containing an excessive amount of fat may give rise to digestive disturbances in children. Thus, in one case of this kind which came under his observation, he found the human milk to contain 16.5 per cent of total solids, and 8 per cent of fat. (See BUDIN: *The nursing*, p. 92.)

milk and buttermilk, the addition of certain alkaline substances to the diet, such as citrates, the dilution of cow's milk with barley broth, etc., or with water containing white of egg, as recommended by Lehmann, and the use of such substances as orange juice, as adjuncts to the infant dietary, are in accord with the ideas herein set

TABLE VI  
MISCELLANEOUS SUBSTANCES.

Sample.	Amount taken. Grams.	Weight of ash. Grams.	Per cent of ash.	N/10 $H_2SO_4$ added. c.c.	N/10 NaOH added. c.c.	Available alkalinity in c.c. of N/10 NaOH.	Available alkalinity of 1 gm. in c.c. of N/10 NaOH.
I. a	3.6074	....	....	5	2.90	2.10	0.5821
I. b	2.8320	0.0206	0.727	5	3.55	1.45	0.5120
I. c	10.8753	....	....	10	3.80	6.20	0.6629
II. a	5.1600	0.0233	0.451	5	2.60	2.40	0.4651
II. b	5.1420	0.0289	0.564	5	3.10	1.90	0.3695
I. White of egg, three determinations. II. Orange juice, two determinations.							

forth. Practically all of these aids to artificial infant feeding, based as they are upon the results of sound practical experience, have for their object either the reduction of the amount of fat in the milk or the addition thereto of mineral matter available for the neutralization of acids resulting from metabolism, or both. In this connection it is interesting to note that the ash of white of egg and orange juice both contain considerably more available alkali than cow's milk, as may be seen from the determinations given in Table VI.

It is also interesting to note in this connection that the young of certain animals whose milk is particularly rich in fat begin, soon after birth, to supplement the available alkali in the mother's milk by a partial vegetable diet; indeed, as has already been pointed out by Bunge, the milk of certain herbivorous animals, such as the guinea-pig, which contains excessive amounts of fat, is probably intended merely as an adjunct to the vegetable diet of the young animal. In these extreme cases the milk supplies the greater part of the fat required by the organism, and the vegetable part of the

diet the other food elements required, or at least the greater part of the mineral matter needed, for its proper assimilation.

In conclusion, I desire to express my thanks to Dr. A. M. Peter for the results of the determinations of the alkalinity of cow's milk given in Table III, and to Dr. H. W. Lawson of the Columbia Hospital of this city for the specimens of human milk used in this work, and to Dr. E. C. Schroeder for certain specimens of cow's milk.







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# A COMPARATIVE STUDY OF THE TEMPERATURE COEFFICIENTS OF THE VELOCITIES OF VARIOUS PHYSIOLOGICAL ACTIONS.<sup>1</sup>

By CHARLES D. SNYDER.

[From the Physiological Institute of the University of Berlin, Germany.]

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## INTRODUCTION.

LIVING processes, for the most part, are undoubtedly caused by chemical changes going on within the living substance itself. But there are, nevertheless, some living phenomena which give little or no evidence of underlying chemical changes. One need only mention the functioning of bone and of elastic connective tissue to illustrate this fact. Other processes still may, or may not, be caused directly by purely physical changes, such as the sepa-

<sup>1</sup> The chief points brought forward in this paper were read before the Seventh International Congress of Physiologists, Heidelberg, August 14, 1907.

ration of the urine from the blood by the kidneys, and the latent period and relaxation phase of muscle action.

Now, simple physical actions are known to have rather characteristic temperature coefficients, and in view of this fact it occurred to the writer that, by comparing these temperature coefficients with those which may be determined of various (obscure) physiological actions, we may be able to arrive at some clue as to which of the non-living actions we are finally to ascribe the more complicated phenomena of the living.

If we believe that any given physiological activity is due to some particular physical change, we need only to determine at which velocities the action proceeds under various temperatures, and then compare these results with the velocities of (probable) physical processes under similar changes of temperature, in order to test for ourselves the correctness of our view.<sup>2</sup>

It was with the purpose of making such a test (and thereby of determining also the value of the idea) that attention was directed to the phenomenon of nerve conduction. Basing the investigation upon data at hand in the literature, it was shown that the temperature coefficient of the velocity of the nerve impulse is of such a magnitude that the phenomenon could not be considered as caused by purely physical action at all, but rather by chemical reaction.<sup>3</sup>

#### TEMPERATURE COEFFICIENTS OF VARIOUS PHYSICAL AND PHYSICO-CHEMICAL PHENOMENA.

Before proceeding further with the considerations of the present paper it would be worth while to make a survey of the temperature

<sup>2</sup> See the author's original communication, *Archiv für Anatomie und Physiologie, Physiol. Abh.*, 1907 (April), p. 113. In this paper the idea of comparing temperature coefficients for possible physical causes underlying physiological actions, as outlined above, was clearly expressed. It was also clearly stated in the abstract of the present paper, as published first at the Congress in Heidelberg, August, 1907, and later in the proceedings of the same which appeared in the various journals and archives of physiology during the fall of the same year. Since that time it is encouraging to note that J. LOEB (*Journal of biological chemistry*, October, 1907) and J. BERNSTEIN (*PFLÜGER'S Archiv*, 1908, cxxii, p. 129) have both thought well enough of the idea to use it as a basis for investigations in their own laboratories.

<sup>3</sup> These results and conclusions have since been fully confirmed by exhaustive experiments made by the author himself, a report of which appears in this journal, 1908, xxii, p. 179.

coefficients of various physical and physico-chemical actions which probably obtain in the living body at one time or another. The following list has been prepared for this purpose. In order to have the coefficients considered here expressed in the same terms, they are all given as quotients of  $k_{t+10}/k_t$ , where the action increases with the temperature. But when the action decreases with the temperature, the quotients are determined from  $k_{t-10}/k_t$ . In any event  $k_t$  represents the constant observed at any temperature,  $t$ , and  $k_{t+10}$  or  $k_{t-10}$  represents the constant observed at a temperature  $10^\circ$  higher or lower than  $t^\circ$ . The quotient is then expressed as  $Q_{10}$  with a plus sign in the one case and a minus sign in the other. For it will be seen that the less the velocity of an action is influenced, the more nearly will the value of  $Q_{10}$  approach unity, and then, that the character of the influence, whether increasing or decreasing the action, may be expressed as falling on the positive or negative side of unity. The value of this usage will be seen also when one considers that a physiological end result may be influenced in a positive direction because of an underlying physical action or condition being influenced (say, by temperature) in a correspondingly rapid negative direction. In any case, where the value of  $Q_{10}$  could be more conveniently arrived at by extra- or inter-polation, the logarithmic formula,  $\left(\frac{k_1}{k_2}\right)^{\frac{10}{t_1-t_2}}$ , was made use of.

TEMPERATURE COEFFICIENTS OF VARIOUS PHYSICAL AND PHYSICO-CHEMICAL PROCESSES, EXPRESSED AS QUOTIENTS<sup>4</sup> FOR DIFFERENCES OF  $10^\circ$ .

	$Q_{10}$
Surface-tension: <sup>5</sup> Benzol, -1.028; water . . . . .	-1.019
Formic acid, -1.027; alcohol, -1.023; ether . . . . .	-1.07
Osmosis: 1 per cent cane sugar, $7^\circ$ - $22^\circ$ , <sup>6</sup> 1.05; $7^\circ$ - $36^\circ$ <sup>7</sup> . . . . .	-1.04
Diffusion: Of $\text{ZnSO}_4$ in water, <sup>8</sup> $1^\circ$ - $48^\circ$ . . . . .	+1.53
Of acetic acid, $8^\circ$ - $14.5^\circ$ , in water <sup>9</sup> . . . . .	+1.59

<sup>4</sup> Where the reaction diminishes with rise of temperature the  $k$  values are reversed in the formula, and the quotient is then indicated as negative.

<sup>5</sup> See WÜLLNER's *Lehrbuch der Physik*, 5te Aufl. 1895, i, p. 410; BRUNNER: POGENDORF'S *Annalen*, 1847, lxx, p. 5; SCHIFF, *Gazetta chimica italiana*, 1884, xiv, p. 379; VAN'T HOFF'S *Vorlesungen über physikalische Chemie*, 1903, iii, p. 70.

<sup>6</sup> See OSTWALD'S *Lehrbuch der allgemeine Chemie*, 2 Aufl., 1891, i, p. 660.

<sup>7</sup> VAN'T HOFF: *Loc. cit.*, ii, p. 32.

<sup>8</sup> WÜLLNER'S *Lehrbuch*, *loc. cit.*, p. 455; OSTWALD'S *Lehrbuch*, *loc. cit.*, p. 683.

<sup>9</sup> In general, however, the rate of diffusion of salts is directly proportional to the change of temperature.

<i>Electric conductivity of solutions:</i> <sup>10</sup> Sat. sol. of NaCl . . . . .	+1.05
Of KCl $n/50$ , 0°-33°, +1.26; KCl $n/10$ , 0°-36° . . . . .	+1.24
<i>Solubility of gases in water between 0° and 45°:</i> <sup>11</sup> Of oxygen (Winkler), -1.24; of CO <sub>2</sub> (Bohr and Bock) . . . . .	-1.32
<i>Solubility of some salts:</i> <sup>12</sup> KCl, +1.08; NaCl, . . . . .	+1.009
Of Na <sub>2</sub> CO <sub>3</sub> between 0°-32.5° . . . . .	+1.95
Of Na <sub>2</sub> HPO <sub>4</sub> between 10° and 45°, +2.33; Na <sub>2</sub> SO <sub>4</sub> between 0° and 32.75° . . . . .	+2.05
<i>Viscosity (absolute internal friction) of fluids:</i> <sup>13</sup> Of water (Thorpe and Rogers), 0°-50° . . . . .	-1.25
Of NaCl solution, 1 per cent (Hosking), 0°-50° . . . . .	-1.25
Of sugar solution, 1 per cent (Hosking), 0°-50° . . . . .	-1.26
Of butyric acid (Thorpe and Rogers), 0°-50° . . . . .	-1.19
Of defibrinated blood, dog (Burton-Opitz), 18°-43° . . . . .	-1.30
Of serum, dog (Burton-Opitz), 15°-40° . . . . .	-1.27
Of ricinus oil (Kahlbaum, Arndt) 2°-42° . . . . .	-2.27
<i>Vapor pressure:</i> <sup>14</sup> Of water, between 19° and 50° . . . . .	+1.99
Of alcohol . . . . .	+1.75
Of NaCl solution, 1 per cent in water . . . . .	+1.7
Of CaCl <sub>2</sub> solution . . . . .	+1.4
<i>Expansion of liquids:</i> <sup>15</sup> Of water, between 4° and 55° . . . . .	+1.003
Of ethyl bromide, between 40° and 80° (Thorpe) . . . . .	+1.09
<i>Elasticity:</i> <sup>16</sup> Of caoutchouc, pure, raw, 19°-45° . . . . .	-1.27
Of caoutchouc, black vulcanized, 21°-60° . . . . .	-1.03
<i>Velocity of sound:</i> <sup>17</sup> In water, 4° to 25° . . . . .	+1.02
In NaCl solution, conc., 15° to 18° . . . . .	-1.018
In air (Greeley), -45.6° to 0.0° . . . . .	+1.018
In dry air (Ciccone and Campanille) -40° to 60° . . . . .	+1.015
<i>Polarization capacities (electrochemical):</i> <sup>18</sup> Of E. M. F. necessary for decomposition of $n/1$ and $n/20$ solutions of H <sub>2</sub> SO <sub>4</sub> ; $n/10$ HCl; $n/10$ and $n/30$ NaOH; $n/2$ and $n/10$ C <sub>2</sub> O <sub>4</sub> H <sub>2</sub> , between 0° and 60°, none higher than . . . . .	-1.001
Of various elements, <sup>19</sup> between 0° and 40°, containing CuSO <sub>4</sub> , ZnSO <sub>4</sub> , CdSO <sub>4</sub> , Cu(NO <sub>3</sub> ) <sub>2</sub> , Pb (NO <sub>3</sub> ) <sub>2</sub> , Ag <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub> , the value of Q <sub>10</sub> varies from -1.01 to . . . . .	-1.02

<sup>10</sup> See LANDOLT, BORNSTEIN, and MEYERHOFFER: *Physikalisch-chemische Tabellen*, 1905, pp. 753-760. The influence of temperature upon migration velocity of ions is about the same as upon the electric conductivity of solutions; upon the transport number of ions it is infinitely small.

<sup>11</sup> LANDOLT's *Tabellen*, *loc. cit.*, p. 599.

<sup>12</sup> OSTWALD's *Lehrbuch*, *loc. cit.*, pp. 1048 ff., and COMEY's *Dictionary of solubilities*, 1896, pp. 92, 263, 311, and 452.

<sup>13</sup> LANDOLT, BORNSTEIN, and MEYERHOFFER: *Physikalisch-chemische Tabellen*, 1905, pp. 77-86; *Zeitschrift für Electrochemie*, 1907, xiii, p. 580.

<sup>14</sup> OSTWALD's *Lehrbuch*, *loc. cit.*, pp. 280, 310, 708.

<sup>15</sup> *Ibid.*, pp. 280 and 282.

<sup>16</sup> LANDOLT's *Tabellen*, *loc. cit.*, p. 43.

<sup>17</sup> *Ibid.*, pp. 774-799.

<sup>18</sup> M. LE BLANC: *Zeitschrift für physikalische Chemie*, 1893, xii, p. 333.

<sup>19</sup> H. JAHN: *Zeitschrift für physikalische Chemie*, 1895, xviii, p. 416.

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Of the total heat quantities at the electrodes of decomposition  
cells,<sup>21</sup> 0°-45° (electrolytes same as above) the highest value  
of  $Q_{10}$  . . . . . -1.03

It may here be repeated that  $Q_{10}$  for chemical reactions usually lies between 2 and 3. In looking over the foregoing list of coefficients it will be noticed that only in a few cases do they reach the magnitude of 2. The coefficient for *vapor pressure* of water is nearly 2. But until we have reason to believe the vapor pressure of water or of aqueous solutions exerts an important influence upon physiological actions we cannot seriously consider it in connection with our problems.

*Diffusion velocities*, on the other hand, are doubtless important factors in physiological processes. Besides the cases mentioned in the list one recalls the case of magnesium hydroxid in benzoic acid, whose temperature coefficient is about 1.5. Nernst<sup>21</sup> showed that, when chemical reaction in a heterogeneous system depends upon the velocity of diffusion of one of the reacting bodies, then the influence of temperature upon the velocity of the chemical reaction becomes a negligible quantity. For the reaction cannot go on faster than the rate of diffusion, and the velocities constants observed will be those of the latter and not of the former process. This is a very important fact to bear in mind in cases of physiological actions which show low temperature coefficients where high ones are expected. In such a case the velocity of the suspected chemical reaction may be masked by the slowness of necessary diffusions. Voigtländer<sup>22</sup> has pointed out that diffusion velocities of most substances in aqueous solution go on in simple proportion to changes of temperature. It is of interest to note, further, that he finds "small quantities of agar-agar dissolved in water make very little difference in the diffusion velocity of the solution."

At present little is known concerning the temperature coefficient of *electro-chemical polarization capacities*. The examples in the list show a low coefficient, but Professor R. Luther in a personal communication tells the writer that there are cases where the value of  $Q_{10}$  may be as high as 2. In this event the ideas of E. du Bois-Reymond may, in one sense, still be right.

<sup>20</sup> *Ibid.*, 1898, xxvi, p. 402.

<sup>21</sup> NERNST: *Zeitschrift für physikalische Chemie*, 1904, lxvii, p. 52.

<sup>22</sup> VOIGTLÄNDER: *Zeitschrift für physikalische Chemie*, 1890, iii, p. 316.

The value of  $Q_{10}$  for *electrical conductivity in dilute solutions* of salts, as seen in the list, is 1.26. It is of interest to note, in passing, that the coefficient of the "velocity of conduction" of Boruttau's<sup>23</sup> artificial-nerve conductor (modification of Hermann's "Kernleiter" apparatus<sup>24</sup>) is 1.36, which is as one would expect for this phenomenon of electrical conductivity in dilute solutions. It is needless to point out that the Hermann-Boruttau apparatus will no longer serve as an artificial nerve, for, as has been shown above, this apparatus must have a temperature coefficient of 2 or 3. In this connection, however, one recalls the experiment of R. Luther,<sup>25</sup> in which he demonstrated a *spatial transmission of a chemical reaction* ( $\text{KMnO}_4$  in  $\text{C}_2\text{O}_4\text{H}_2 + \text{H}_2\text{SO}_4$ ). The rate of transmission of this reaction is measurable, and one naturally wonders what the effect of temperature would be upon its velocity, etc.

The temperature coefficient of the *viscosity* of ricinus oil is, as would be expected of an oil, very high. But the viscosity of the body fluids undoubtedly have temperature coefficients more nearly like those which can be calculated from Burton-Opitz's observations on blood plasma and serum, and for water and a 1 per cent solution of NaCl. The value of  $Q_{10}$  in all of these cases is nearly 1.3. As will be seen later, we shall have occasion to give this action further consideration.

The temperature coefficient of the *solubility of some salts*, such as KCl, NaCl,  $\text{CaCl}_2$ , is very small. It is, however, worthy of note that the coefficients for a few of them ( $\text{Na}_2\text{CO}_3$ , between  $0^\circ$  and  $32.5^\circ$ ;  $\text{Na}_2\text{SO}_4$ , between  $0^\circ$  and  $32.75^\circ$ ; and  $\text{Na}_2\text{HPO}_4$ , between  $10^\circ$  and  $45^\circ$ ) are of the magnitude of chemical reactions. However, since we do not have these salts in saturated solutions in living tissues their rapid solubilities cannot directly explain the phenomena of the latter.

Surface tension, osmosis, absorption, internal friction, electrical conductivity, dielectric capacities, migration velocity and transport number of ions, and the solubility of gases, are all factors which doubtless are important in the functioning of physiological processes. But from the data at hand it will be seen that *temperature influences the velocities of these actions only to a slight degree*. In the

<sup>23</sup> BORUTTAU: Archiv für die gesammte Physiologie, 1894, lviii, p. 54.

<sup>24</sup> HERMANN: Handbuch der Physiologie, ii, p. 174.

<sup>25</sup> R. LUTHER: Zeitschrift für Electrochemie, 1906, Nr. 32, p. 596.



cases of dielectric capacities and migration velocity of ions the temperature coefficient is infinitely small.<sup>26</sup>

The object of the present study is to apply the method, as outlined above, to a few other physiological phenomena, namely, to the duration of the latent phase, the shortening phase, and the relaxation phase, as well as to the whole period of muscle action,<sup>27</sup> also to the latency period of the extrinsic nerves of the heart.

#### THE LATENT PHASE OF MUSCLE ACTION.

*a. Of smooth muscle.* — From the exhaustive work of P. Schultz<sup>28</sup> and also of C. C. Stewart<sup>29</sup> — the former working with the smooth muscle of frog's stomach, the latter with smooth muscle from the cat's bladder — the temperature coefficient for the latent period is found to be quite steadily between 2 and 3. This coefficient holds good between 0° and 35° C.

*b. Of cross-striated muscle.* — On the other hand, from the very complete work of three very reliable investigators the temperature coefficient for the latent period of cross-striated muscle of the frog is quite as constantly 1.6.<sup>30</sup> The muscles were curarized in some cases, in others they were not curarized. The coefficient holds good between the temperatures of -2.5° and 28° C.

*c. Of cardiac muscle.* — Few observations have been made on the velocity of the latent period of cardiac muscle at different temperatures. From observations of Marchand<sup>31</sup> the coefficient seems to be as high as 2, from those of Bornstein<sup>32</sup> in the frog ventricle between 9° and 30° it seems to be as low as 1.4. This point requires further investigation.

<sup>26</sup> LANDOLT's Tabellen, *loc. cit.*, pp. 753-769.

<sup>27</sup> In this paper the term "muscle action," recommended by TH. W. ENGELMANN, will be adopted (see his paper "Zur Theorie der Contractilität," Sitzungsberichte der königlichen preussischen Akademie der Wissenschaften, 1906, xxxix, p. 720. This term will connote the three "phases," mentioned above, taken together.

<sup>28</sup> P. SCHULTZ: Archiv für Anatomie und Physiologie, Physiol. Abt., 1897, p. 22.

<sup>29</sup> C. C. STEWART: This journal, 1901, iv, see footnote on p. 202.

<sup>30</sup> TIGERSTEDT: Archiv für Anatomie und Physiologie, Physiol. Abt. Suppl., 1885, p. 111; YEO: Journal of physiology, 1888, ix, p. 396; GAD and HEYMANS: Archiv für Anatomie und Physiologie, Physiol. Abt., Suppl., 1890, p. 59.

<sup>31</sup> MARCHAND: Archiv für die gesammte Physiologie, 1877, xv, p. 515.

<sup>32</sup> BORNSTEIN: Archiv für Anatomie und Physiologie, Physiol. Abt. Suppl., 1906, p. 343.

## THE DURATION OF THE SHORTENING PHASE OF MUSCLE ACTION.

We next turn our attention to the shortening, or contraction, phase of muscle action. From Gad and Heyman's<sup>33</sup> work on frog's gastrocnemius the coefficient is 2.4 between  $-4^{\circ}$  and  $40^{\circ}$ . From the experiments of Yeo and Cash<sup>34</sup> on frog's gastrocnemius, of R. Magnus<sup>35</sup> on muscle from small intestine of mammals, of C. C. Stewart<sup>36</sup> on smooth muscle from cat's bladder, and P. Schultz<sup>37</sup> on smooth muscle from frog's stomach, we find a temperature coefficient of very nearly 2.0. This holds pretty well for cross-striated muscle between  $6^{\circ}$  and  $27^{\circ}$  C., and for cat's bladder muscle between  $15^{\circ}$  and  $40^{\circ}$ . The experiments on mammalian intestine were limited (for this phase) to the range from  $5^{\circ}$  to  $12^{\circ}$ , and show a coefficient more nearly 3 than 2.

From the writer's own experiments<sup>38</sup> on frog heart the value of  $Q_{10}$  for the shortening phase (systole) for cardiac muscle lies quite clearly between 2 and 3. This is true not only for sinus, but also for ventricle muscle of the frog's heart. From the constants observed by Burdon-Sanderson<sup>39</sup> this coefficient is more nearly 2 than 3 (see tables under the next heading).

## THE RELAXATION PHASE OF MUSCLE ACTION.

It is in the realm of the relaxation phase that we meet constants that are decidedly irregular. From the writer's experiments this coefficient for cardiac muscle varies from 1.1 to 6.0, and even more than this for the extremes of temperature. This great variation is due to the fact that the constants were all taken from automatically beating tissues where great difficulty is met with in separating the relaxation phase from the "pause," (if such really exists) on the one hand and the "pause" from the latent period on the other.

<sup>33</sup> GAD and HEYMANS: *Loc. cit.*, See "Tafel iv," curve "Da."

<sup>34</sup> YEO and CASH: *Journal of physiology*, 1883, iv, p. 213, Table IV.

<sup>35</sup> R. MAGNUS: *Archiv für die gesammte Physiologie*, 1904, cli, p. 123.

<sup>36</sup> P. SCHULTZ: *Loc. cit.*

<sup>37</sup> *Loc. cit.*, p. 22. See Fig. 4.

<sup>38</sup> SNYDER: *Archiv für Anatomie und Physiologie, Physiol. Abt.*, 1907, p. 118. During these experiments a large number of tracings were taken with a rapidly moving drum, from which the data for this calculation could easily be made.

<sup>39</sup> BURDON-SANDERSON: *Journal of physiology*, 1880, ii, p. 384.

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As the data on this point have not yet been published, the following tables taken from the protocols of the experiments referred to above will serve as examples. The data under the heading "diastole" in the last two columns show the great variations of

Temperature coefficient of 2 phases of <i>sinus muscle</i> action.	Temperature in degrees C.	Systole (contraction phase).		Diastole (relaxation phase).	
		Duration in seconds.	$Q_{10}$	Duration in seconds.	$Q_{10}$
Of frog's heart . . . . .	15.8	0.695		0.64	
First exp., Feb. 4, 1907 . .	25.0	0.393	1.8	0.53	1.2
	16.7	0.612	1.7	0.55	1.0
	3.0	2.66	2.9	4.00	6.2
	8.5	2.0		1.08	
	15.0	1.13	2.4	0.71	1.9
	27.0	0.40	2.4	0.57	1.2
Next morning, same sinus .	17.2	1.34	3.3	0.39	1.5
	2.0	4.0	2.0	5.0	6.0
	28.0	0.3	2.7	0.7	2.1
Second exp., Feb. 12, 1907 .	16.9	0.7		0.72	
	7.0	1.8	2.5	2.00	2.7
	0.9	3.6	3.1	5.95	6.0
	14.5	1.2	2.2	1.1	3.4
Next morning, same sinus .	18.3	0.5		0.8	
	8.3	1.45	2.9	1.9	2.3
Average value of $Q_{10}$ . . .			2.5		3.0

this coefficient. In passing, one may notice the greater regularity of the coefficient of the shortening phase, or systole.

The literature contains meagre and incomplete studies of temperature influence on the relaxation phase of cross-striated muscle. From the data of Yeo and Cash<sup>40</sup> we get a coefficient of 1.9 between 7° and 18° C. Above 18° temperature seems to have no influence upon the duration of the relaxation phase.

<sup>40</sup> YEO and CASH: Journal of physiology, 1883, iv, p. 213.

From C. C. Stewart's work on cat's bladder, however, we get a very constant coefficient of 1.4 between the wide range of 15° to 40° C.<sup>41</sup>

Temperature coefficient of 2 phases of <i>ventricular</i> muscle action.	Tempera- ture in degrees C.	Systole.		Diastole.	
		Duration in seconds.	$Q_{10}$	Duration in seconds	$Q_{10}$
First exp., Feb. 5, 1907 . .	20.0	0.56		0.36	
	26.5	0.42	1.6	0.34	1.1
	2.1	2.7	2.1	4.9	4.7
	11.5	1.34	2.1	1.7	2.9
			2.0		2.8
	0.0	3.0		5.5	
Second exp., Feb. 6, 1907 .	19.5	0.6		0.46	
	13.5	1.2	3.2	2.4	2.8
	26.0	0.4	2.4	0.4	4.2
Third exp., Feb. 15, 1907 .	15.0	1.3		2.5	
	4.5	3.2	2.4	5.3	2.0
	1.5	3.8		5.3	
	14.5	1.45	2.1	2.3	2.8
Average value of $Q_{10}$ . .			2.2		2.9

One naturally thinks of the relaxation of muscle as being a passive action, and therefore one which could not be greatly affected by temperature. The data now at hand (see Fick) are sufficient to show that this phase of muscle action cannot be passive. The many high coefficients, furthermore, suggest that chemical or, more specifically and more probably, ferment action is involved.

#### THE DURATION OF THE WHOLE MUSCLE ACTION.

From the observations made upon rhythmically beating cardiac and smooth muscle the temperature coefficient of the duration of the whole muscle action lies, quite clearly, between 2 and 3.<sup>42</sup> The

<sup>41</sup> *Loc. cit.*

<sup>42</sup> SNYDER: Archiv für Anatomie und Physiologie, Physiol. Abt., 1907, pp. 118, 126.

change in tone in smooth muscle,<sup>43</sup> and the greatly prolonged plateau in the last third of the relaxation phase of contractions caused by artificial stimuli make the observations of temperature effects upon cardiac and smooth muscle difficult of interpretation.

Some experiments on cross-striated muscle<sup>44</sup> seem to show a coefficient of 2 for the whole muscle action. For example, from the hyoglossus muscle of the frog we have

At 7°, total time of whole muscle action . .	77.5
At 30°, total time of whole muscle action . .	18.0
Value of $Q_{10} = 2$ .	

On the other hand, the gastrocnemius muscle of the frog gives the following:

At 9°, total time of whole muscle action . . .	24
At 28°, total time of whole muscle action . .	10
Value of $Q_{10} = 1.5$ .	

This section must not be closed without referring to the influence of temperature upon the electrical response to stimulation of muscle as determined by Burdon-Sanderson.<sup>45</sup> Using curarized sartorius muscles of the frog and photographing the excursions of the capillary electrometer due to electrical stimulation of the muscle, this writer found the following for fresh muscle (page 347):

At 17°, excursion per second . . .	32 cm.
At 14°, excursion per second . . .	26 "
At 9°, excursion per second . . .	19 "
At 7°, excursion per second . . .	14 "
From these constants $Q_{10} = 2.3$ .	

For muscle kept twenty-four hours in salt solution, these:

At 20°, excursion per second . .	22.0 cm.
At 18°, excursion per second . .	20.0 "
At 14°, excursion per second . .	14.0 "
At 9°, excursion per second . .	9.0 "
At 6°, excursion per second . .	7.5 "
From which constants $Q_{10} = 2.2$ .	

#### SUMMARY AND DISCUSSION OF MUSCLE-NERVE ACTIVITIES.

If we put the characteristic coefficients of the various phases of muscle action, as found in the foregoing pages, in a form where we may compare them, we find a curious relation to exist:

<sup>43</sup> P. SCHULTZ: *Loc. cit.*

<sup>44</sup> From BRODIE'S *Essentials of experimental physiology*, 1898, pp. 45 and 47; see also GAD and HEYMANS: *Loc. cit.*

<sup>45</sup> BURDON-SANDERSON: *Journal of physiology*, 1898, xxiii, p. 325.

	Latent period.	Shortening phase.	Relaxation phase.
Smooth muscle . . . . .	2.7	2.0	1.4
Cross-striated muscle . . .	1.6	2.0	1.9
Cardiac muscle . . . . .	1.4 (?)	2.3	3.0 (?)

For smooth muscle the highest coefficient occurs during the latent period, the lowest during the relaxation phase. For cardiac muscle the lowest coefficient occurs during the latent period, the highest during the relaxation phase, thus reversing the relations which exist in the case of smooth muscle. The coefficients for cross-striated muscle seem to occupy a middle position. For reasons already stated, however, the coefficient of the latent period of cardiac muscle is not to be relied upon altogether, and until it is determined beyond doubt, too much stress must not be laid upon this matter. It will also be remembered that the individual determinations of the coefficient for the diastole of sinus muscle showed variations between 1 and 6. This average coefficient of heart muscle therefore is not to be looked upon yet as being characteristic. Accordingly I have marked both of these latter cases with an interrogation point.

From the foregoing determinations of temperature coefficients we may come to the following conclusions:

1. The shortening phase of all kinds of muscle is a period when chemical change is taking place.<sup>46</sup>
2. The latent period of smooth muscle and probably the relaxation phase of cardiac muscle are also periods of chemical change.
3. The relaxation phase of smooth muscle and probably the latent period of cardiac muscle are periods of time when purely physical change is taking place.
4. The latent period of cross-striated muscle is most probably a period of purely physical action. But if it be of a chemical nature the process may involve a kind of reaction either where hydration processes, or electro-chemical polarization is affected.

If we admit that purely physical action is taking place during the relaxation of smooth muscle (and also during the latent period

<sup>46</sup> The temperature coefficient of ENGELMANN'S "artificial muscle" can be calculated from his observations on the effect of temperature in producing contractions. On pages 711 and 713 of his paper, "Zur Theorie der Contractilität," we find constants for strips of caoutchouc. The temperature coefficient according to these constants is *ca.* 1.04.

of cardiac muscle), the question at once arises, What physical action can it be? What physical processes have temperature coefficients equal to 1.4, or nearly so? Turning to our list, we find the following:

Vapor pressure of $\text{CaCl}_2$ solution . . . . .	+1.4
Elasticity of soft rubber . . . . .	-1.3
Diffusion of $\text{ZnSO}_4$ and of acetic acid . . . . .	+1.6
Electric conductivity of $\pi/10$ KCl . . . . .	+1.24
Solubility of $\text{CO}_2$ . . . . .	-1.32
Viscosity of dog's defibrinated blood . . . . .	-1.3

The solubility of gases in the body liquids, the viscosity of the muscle fluids, elasticity of muscle tissue, electric conductivity of electrolytes, may then, one or all, constitute the physical action underlying the relaxation of smooth muscle and the latent period of cardiac muscle. That here we may be dealing with electrical phenomena is suggested further by the following considerations.

It has already been said that further experiments on the temperature velocities obtained from a system something like Hermann's "Kernleiter" apparatus, ought to yield most interesting results. With this apparatus Boruttau<sup>47</sup> already has shown "dass die Fortpflanzungsgeschwindigkeit mit steigender Temperatur zu- und mit sinkender, abnimmt." His constants, for example, were:

At 10° C., velocity per second . . . . .	100 m.
At 26° C., velocity per second . . . . .	163.5 m.
$Q_{10}$ from these constants is 1.36!	

We now know that this coefficient is too low to be considered in connection with nerve conduction, as Boruttau intended it; but it is a remarkable fact that here we have a number practically the same as that of the relaxation phase of smooth muscle, and of the latent period of heart muscle, so far as we can be sure of the latter. These phenomena may therefore be electrical effects similar to those occurring in the artificial nerve conductor.

The temperature coefficient of the "Kernleiter" may also explain the low coefficient of the latent period of cross-striated muscle. But in addition to this we have the physical coefficients of diffusion, 1.6 (in isolated cases<sup>48</sup>) and of the vapor pressure of 1 per cent NaCl solution, 1.7.

<sup>47</sup> BORUTTAU: Archiv für die gesammte Physiologie, 1898, lviii, p. 54.

<sup>48</sup> For example, the diffusion of magnesia in NERNST's experiments. See Zeitschrift für physikalische Chemie, 1904, xlvii, p. 52.

One cannot help thinking, too, that the solubility of some ion-proteid may come into play. If  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{SO}_4$ , and  $\text{NaH}_2\text{PO}_4$  have such high solubility temperature coefficients just at the temperatures normal for living tissues (that is, coefficients of 1.9, 2.0, and 2.3 respectively), then one is naturally inclined to think that some sodium-proteid salt, or salts, may possibly be present in saturated solution in the body tissues, and with changes of temperature suffer changes in their solubilities as do their inorganic analogues, — that is, precipitate upon lowering, and go into solution upon raising, the temperature.

The work of Mellanby,<sup>49</sup> although not exhaustive for various proteids, however, so far as it goes, does not give support to this idea. Mellanby finds that globulin dissolves in normal solutions of NaCl at various temperatures somewhat as follows (from page 353):

In about 68 parts of a normal solution of NaCl,  
 At 15° . . . 37 per cent of the globulin is dissolved.  
 At 20° . . . 57 per cent of the globulin is dissolved.  
 $Q_{10} = 1.27$ .

In about 41 parts of a normal solution of NaCl,  
 At 23° . . . 26.5 per cent of the globulin dissolves.  
 At 40° . . . 51.0 per cent of the globulin dissolves.  
 $Q_{10} = 1.39$ .

The solubility curves of  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{SO}_4$ , and of  $\text{NaH}_2\text{PO}_4$ , however, have critical points in them (due to hydration changes) at just those temperatures where the phenomena of maximum activity, of heat injury (*Wärme-Lähmung*) and heat rigor occur in animal tissues (Fig. 1). This fact therefore still leads one to believe in the existence of saturated solutions of ion-proteids in spite of the contra-indication of Mellanby's results.

In every case, however, transmission of sound waves, solubility of KCl and NaCl, electric conductivity of NaCl, osmosis and surface-tension phenomena, as far as we now know them, are all, on account of their low temperature coefficients, equally excluded from consideration as important primary processes which influence the *velocity* in any one of the recognized phases of muscle activity.

<sup>49</sup> MELLANBY: *Journal of physiology*, 1905, xxxiii, p. 338.



# THE TEMPERATURE COEFFICIENT OF THE EXTRINSIC NERVES OF THE HEART.

It is well known that a considerable period of time intervenes between the point of stimulus and the moment when the effect of stimulating the extrinsic nerves of the heart takes place. Experiments show that this latency of the heart nerves is influenced by

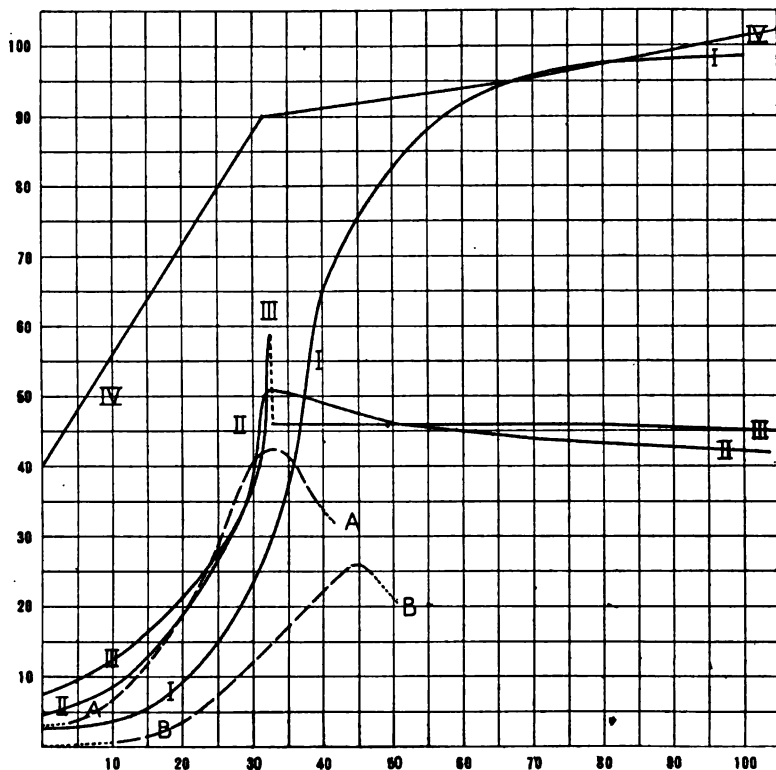


FIGURE 1.—Heart curves for *A*, rate of terrapin's ventricle; *B*, rate of cat's heart compared with solubility curves for *I*,  $\text{Na}_2\text{HPO}_4$ ; *II*,  $\text{Na}_2\text{SO}_4$ ; *III*,  $\text{Na}_2\text{CO}_3$ ; *IV*,  $\text{Sr}_2(\text{NO}_3)_2$ . The ordinates give temperature, the abscissæ give grams of dissolved substance in one hundred parts of water.

temperature. From the observations of Baxt<sup>50</sup> and of G. N. Stewart<sup>51</sup> I find the coefficients to be as shown in the following tables.

The coefficient of latency in the accelerans nerve of the dog and

<sup>50</sup> BAXT: *Berichte der königlichen sächsischen Gesellschaft der Wissenschaften, Math. phys. Klasse*, 1875.

<sup>51</sup> G. N. STEWART: *Journal of physiology*, 1892, xiii, p. 59.

of the sympathetic of the frog, it will be noted, is as high as that of chemical reactions. The latency of the frog's vagus could only be determined for temperatures between  $21^{\circ}$  and  $39^{\circ}$ , and for a limited number of observations. At these temperatures the coefficient is decidedly like those for physical processes. Baxt's observations unfortunately contain no data on the vagus nerve from which a temperature coefficient could be calculated. The work done in Professor Howell's laboratory for a number of years past on the relation existing between potassium and vagus inhibition<sup>52</sup> would lead us to expect to find that important chemical changes occur during the latent period of vagus stimulation. The influence of temperature upon the velocity of this latency should therefore be redetermined for as wide a range of temperatures as possible. The results would doubtless show that the coefficient is equally as high as it is for the latent period of accelerans stimulation.

Recently O. Frank<sup>53</sup> has published results of experiments similar to those of Baxt. Unfortunately no record of latency at various temperatures are to be found in this paper.

However the rates of heart beat before and during stimulation are given. While the results do not enable us to calculate a coefficient for latency, they are still of some interest in this connection. From them we can see what the temperature coefficient of the heart rate is before and during stimulation. This is shown in the table.<sup>54</sup>

In the case of the rabbits the coefficient of the heart's frequency is as high as that of chemical action, both before and during stimulation of the vagus, the latter, however, being lower than the former.

In the case of the dogs we have the same relation of the coefficient magnitudes, the coefficient before stimulation being greater than that during stimulation. But the absolute magnitudes are different from those of the rabbits. For the dogs the coefficient before is again like that of chemical action while that during

<sup>52</sup> See E. G. MARTIN: The inhibitory influence of potassium chloride on the heart, and the effect of variations of temperature upon this inhibition and upon vagus inhibition, *This journal*, 1904, xi, p. 370. HOWELL and DUKE, Inorganic salts and cardiac nerves, *Journal of physiology*, 1906, xxxv, p. 131; and now, as this paper goes to print, HOWELL and DUKE on The liberation of K salts in heart tissues by stimulation of vagus, *This journal*, 1908, xxi, p. 51.

<sup>53</sup> O. FRANK: *Zeitschrift für Biologie*, 1907, xxxi, p. 392.

<sup>54</sup> Since this paper was read I find that A. KANITZ (*PFLÜGER'S Archiv*, 1907, cxviii, p. 601) has also calculated temperature coefficients from FRANK's results.

stimulation of the vagus is doubtful, two of the four coefficients being as low as we found them to be for Boruttau's experiments on the artificial nerve conductor.

TEMPERATURE COEFFICIENT OF THE EXTRINSIC NERVES OF THE HEART; THEIR LATENCY TO ELECTRICAL STIMULUS.

Remarks.	$t_1$ .	$t_2$ .	$k_1$ .	$k_2$ .	$\left(\frac{k_2}{k_1}\right)^{\frac{10}{t_1-t_2}}$ .
1.					
Accelerans $n$ . of the dog. <i>Baxt.</i> 1875.	A { 39.43	34.85	7	13.0	3.9
	39.43	36.0	7	10.0	2.8
	37.48	34.85	9	13.0	4.1
$\frac{1}{2} \mu_A = 12,960$ .	B { 42.43	37.3	6	9.0	2.2
	39.35	37.3	7	9.0	3.4
$\frac{1}{2} \mu_B = 7,750$ .	42.43	39.35	6	7.0	1.7
					Av. 3.0
2.					
Accelerans $n$ . of the dog. <i>Baxt.</i> 1875.	A { 38.4	29.7	7	15.0	2.4
	36.1	27.8	9	22.0	2.9
	38.4	32.5	7	12.0	2.5
	36.1	29.7	9	15.0	2.2
	38.4	27.8	7	22.0	2.9
Value of $\mu$ for A and B = 16,265.	B { 38.1	27.6	9	17.5	1.9
	34.2	27.6	12	17.5	1.8
	32.8	27.6	13	17.5	1.7
	38.1	30.6	9	15.0	2.0
	30.6	27.6	15	17.5	1.7
					Av. 2.2

NOTE. — The values of  $k_1$  and  $k_2$  in the above columns represent the duration of time in seconds intervening between the moment of stimulus and the moment when this stimulus shows its effect upon the action of the heart. The meaning of the  $\mu$  value is explained further on.

LATENCY PERIOD OF THE EXTRINSIC NERVES OF THE HEART REACTING TO  
ELECTRICAL STIMULI.

Remarks.	$t_1$ .	$t_2$ .	$k_1$ .	$k_2$ .	$\left(\frac{k_2}{k_1}\right)^{\frac{10}{t_1-t_2}}$ .
The sympathetic n. of the frog's heart. <i>G. N. Stewart</i> , 1892.  $\mu = 14,990$ .	8.0	6.0	6.0	7.0	2.2
	8.0	3.5	6.0	10.5	3.5
	12.3	8.0	5.6	6.0	1.2
	18.3	12.3	4.0	5.6	1.8
	12.3	3.5	5.6	10.5	2.0
	18.3	3.5	4.0	10.5	1.9
	18.3	8.0	4.0	6.0	1.5
	18.3	6.0	4.0	7.0	1.6
					Av 2.0

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TEMPERATURE COEFFICIENT OF THE HEART RATE BEFORE AND AFTER STIMULATION OF THE VAGUS NERVE. (FROM THE OBSERVATIONS OF OTTO FRANK.)

Remarks.	Tempera- ture in degrees C.	Before stimulation of vagus.		During stimulus of vagus n.	
		Rate per sec.	Q <sub>10</sub> .	Rate per sec.	Q <sub>10</sub> .
Experiment 1 (rabbit)	37.0	4.62	2.8	. . .	....
	27.0	1.64		....	
Experiment 2     "	37.6	4.80	3.1	3.50	2.6
	27.7	1.56		1.33	
	20.7	0.55		0.55	
Experiment 3     "	38.2	5.00	3.1	1.7	1.8
	24.8	1.12		1.09	
	18.7	0.53		0.55	
Experiment 4     "	37.6	4.70	2.7	3.10	2.2
	24.9	1.46		0.77	
	19.3	0.78		0.75	
	20.3	0.90	2.9	0.83	2.9
	29.0	2.25		2.10	
		Av. 3.18		Av. 2.6	
Experiment 1 (dog)	34.9	1.84	2.2	0.42	1.4
	21.6	0.65		0.26	
Experiment 2     "	34.8	1.78	1.9	0.40	1.4
	18.0	0.63		0.23	
Experiment 3     "	36.6	2.61	1.9	1.42	2.0
	25.0	1.22		0.64	
Experiment 4     "	30.9	2.0	2.6	1.24	2.3
	18.7	0.62		0.45	
		Av. 2.15		Av. 1.78	

## CONCLUDING REMARKS.

In view of the fact that so many physiological actions have temperature coefficients equal to those of chemical reactions, it is of considerable interest to compare the observed constants with those which may be calculated from an empirical formula representing a similar curve of velocity.

Such a formula has been suggested and repeatedly used by Arrhenius,<sup>55</sup> and is as follows:

$$\text{nat. log. } \frac{k_1}{k_0} = \frac{\mu}{2} \left( \frac{T_1 - T_0}{T_1 T_0} \right).$$

The constants calculated according to this formula for the latent period of smooth muscle from the frog's stomach, for example, and compared with the observed constants agree most satisfactorily. This is shown in the following table, where the observed constants are taken from the work of P. Schultz. The value of  $\mu$  here is 12,760.

Temperature. in degrees C.	$k$ , observed.	$k$ , calculated.
0	5.1	6.8
5	4.0	4.0
10	2.9	2.8
15	2.0	1.8
20	1.5	1.2
25	1.0	0.8
30	0.6	0.6
35	0.3	0.4
40	0.2	0.3

The average value of  $\mu$  for purely chemical reactions, it may be added, is about 13,500.

In the following conspectus I have brought together the chief results of all the temperature determinations which so far have

<sup>55</sup> ARRHENIUS: *Immunochemie*, Leipzig, 1907.

been made of physiological activities. In each case, besides the name of the observer, year of publication, and range of temperature under which the observations were made, I have set down both the average van't Hoff coefficient,  $Q_{10}$ , and also the value of the Arrhenius  $\mu$  as could be calculated from the data.

A CONSPECTUS OF THE TEMPERATURE COEFFICIENTS OF THE VELOCITIES OF VARIOUS PHYSIOLOGICAL ACTIONS.

Kind of activity.	Temperature constants observed by.	In the year.	For a range of temperature in degrees.	Temp. coef. expressed as $Q_{10}$ .	Temp. coef. expressed in value of $\mu/1000$ .	The $\mu$ value expressed by the next nearest multiple of 4.
<i>Rate of heart beat:</i>						
Pacific terrapin, isolated, ventricle . . .	SNYDER	1905	2-32	2.5	16.0	16.0
"Tortoise," <i>in situ</i> , ventricle . . . . .	G. N. STEWART	1892	6-37	2.6	15.7	16.0
Rana escul., isolated, ventricle . . . . .	SNYDER	1907	13-24	2.8	13.4	12.0
Rana escul., isolated, sinus only . . . . .	SNYDER	1907	1-25	2.3	15.8	16.0
Phyllorhoë (mollusca), <i>in situ</i> . . . . .	SNYDER	1906	16-30	2.5	10.7	12.0
Maia verrucosa, isolated . . . . .	SNYDER	1906	7-26	3.0	17.1	16.0
Cat, surviving organ; pulmon. circulation .	{ MARTIN and APPELGARTH	1890	22-40	2.5	16.8	16.0
Cat, surviving organ; artif. circulation . .	LANGENDORFF	1897	10-46	2.3	16.1	16.0
Dog, surviving organ; pulmon. circulation	H. N. MARTIN	1883	28-43	2.8	16.4	16.0
Dog, heart, <i>in situ</i> . . . . .	BAXT	1875	27-42	2.8	13.4	12.0
Rabbit, ventricle . . . . .	{ LANGENDORFF and LEHMANN	1906	37-48	2.4	17.1	16.0
Rabbit, heart <i>in situ</i> . . . . .	WINTERSTEIN	1904	17-42	2.2	13.1	12.0
Man, normal pulse . . . . .	DAVY	1842	36-37	2.3	22.5	24.0
Man, fever pulse . . . . .	LIEBERMEISTER	1875	37-42	2.2	16.7	16.0



Rate of spon. beating, vent. strip in $m/8$ NaCl	SNYDER	1907	12-24	2.0	12.7	12.0
Rate of spon. beating, frog's gullet . . . .	STILES	1901	7-27	2.5	18.8	20.0
Rate of spon. beating, frog's gullet . . . .	SNYDER	1907	16-28	2.5	16.0	16.0
Rate of spon. beating, small intest., mammalian	R. MAGNUS	1904	16-42	2.8	17.4	16.0
<i>Velocity of nervous conduction:</i>						
Of olfactory nerve of the pike . . . . .	NICOLAI	1901-05	3-25	2.6	15.8	16.0
Of motor nerves of the frog . . . . .	V. MIRAM	1906	15-35	1.9	9.5	8.0
Of motor nerves of the frog . . . . .	V. HELMHOLTZ	1850	0-21	3.1	12.6	12.0
<i>Latent period of muscle action:</i>						
Of smooth muscle, cat's bladder . . . . .	C. C. STEWART	1901	10-40	2.7	16.8	16.0
Of smooth muscle, sm. intest., mam. . . .	R. MAGNUS	1904	5-10	6.1	28.9	28.0
Of smooth muscle, frog's stomach . . . .	P. SCHULTZ	1897	0-40	2.5	12.7	12.0
Of $x$ -striated m., frog's gastrocn. . . . .	YEO and CASH	1883	7-34	1.3	4.6	4.0
Of $x$ -striated m., frog's gastrocn. . . . .	TIGERSTEDT	1885	18-29	1.7	8.5	8.0
Of $x$ -striated m., frog's gastrocn. . . . .	YEO	1888	14-28	1.7	8.8	8.0
Of $x$ -striated m., frog's gastrocn. . . . .	GAD and HEYMANS	1890	-2.5-30	1.7	7.7	8.0
Of cardiac muscle, frog . . . . .	BORNSTEIN	1906	9-30	1.4	6.2	8.0
<i>Shortening phase of muscle action:</i>						
Of $x$ -striated muscle, frog . . . . .	YEO and CASH	1883	6-27	2.0	12.0	12.0
Smooth muscle, cat's bladder . . . . .	C. C. STEWART	1901	5-40	2.0	14.3	16.0
Smooth muscle, sm. intest., mam. . . . .	R. MAGNUS	1904	5-13	2.4	17.8	16.0
Cardiac muscle, frog's sinus . . . . .	SNYDER	1907	1-28	2.5	16.0	16.0
Cardiac muscle, frog's ventricle . . . . .	SNYDER	1907	0-26	2.2	14.5	16.0

TABLE (continued).

Kind of Activity.	Temperature constant observed by.	In the year.	For a range of temperature.	Temp. coef. expressed as $Q_{10}$ .	Temp. coef. expressed in $\mu/1000$ .	The $\mu$ value expressed by the next nearest multiple of 4.
<i>Relaxation phase of muscle action:</i>						
Of cross-striated m., frog's gastroc. . . .	YEO and CASH	1883	7-18	1.9	10.6	12.0
Of smooth muscle, cat's bladder . . . .	C. C. STEWART	1901	15-40	1.3	7.2	8.0
Of cardiac muscle, frog's sinus . . . .	SNYDER	1907	1-28	3.0	16.5	16.0
Of cardiac muscle, frog's ventricle . . . .	SNYDER	1907	0-26	2.9	16.4	16.0
<i>Whole muscle action:</i>						
Of x-striated m., frog's gastroc. . . . .	YEO and CASH	1883	7-27	2.0	10.6	12.0
Of x-striated m., frog's gastroc. . . . .	GAD and HEYMANS	1890	-4-40	2.5	15.0	16.0
Of smooth m., frog's stomach . . . . .	P. SCHULTZ	1897	0-45	2.0	10.9	12.0
Of smooth m., cat's bladder . . . . .	C. C. STEWART	1901	20-40	1.7	8.1	8.0
<i>Heart's refractory period:</i>						
Frog . . . . .	BURDON-SANDERSON	1880	12-27	1.8	10.4	12.0
<i>Latency of heart nerves:</i>						
Of accelerans n. of dog . . . . .	BAXT	1875	27-42	2.6	18.5	20.0
Of sympathetic n. of frog . . . . .	G. N. STEWART	1892	3-18	2.0	10.5	12.0

<i>Other activities:</i>						
Expiration of CO <sub>2</sub> by plants . . . . .	CLAUSEN <sup>1</sup>	1890	0-40	2.6	18.5	20.0
Assimilation of CO <sub>2</sub> by plants . . . . .	MATTHAEI <sup>1</sup>	1905	-5-37	3.5	16.6	16.0
Production of C <sub>2</sub> H <sub>5</sub> OH by yeast . . . . .	AHERSON <sup>1</sup>	1903	15-32	2.6	15.6	16.0
Cell-division in Echinoderm eggs . . . . .	K. PETERS <sup>1</sup>	1906	10-24	2.1	12.8	12.0
Development of frog eggs . . . . .	O. HERTWIG <sup>1</sup>	1898	6-24	2.7	16.6	16.0
Pulsating vacuoles of <i>Glaucoma colpidium</i>	A. KANITZ <sup>1</sup>	1907	7-29	2.6	....	....
Rate of respiration of Marmotte . . . . .	DUBOIS	1896	11-36	2.5 <sup>3</sup>	14.5	16.0
Rate of heart beat of Marmotte . . . . .	DUBOIS	1896	11-36	2.2 <sup>3</sup>	12.4	12.0

NOTE. — The tendency for the  $\mu$  values to fall into multiples of 4 is undoubtedly due to the invariable factors 2 and 2.3 used in the Arrhenius formula. It should be said here also that the  $Q_{10}$  value is an *average value*, while the  $\mu$  value holds good for the extreme temperatures indicated in the fourth column.

<sup>1</sup> The temperature coefficients in these cases were first calculated by VAN'T HOFF, KANITZ, AHERSON, PETERS, COHEN, and KANITZ, respectively.

<sup>3</sup> These coefficients hold only for constants at 11° when the animal is in complete sleep compared with those for 36° after the animal has become fully awakened.

In this conspectus are shown coefficients calculated from about 55 different series of experiments, and for about 20 different physiological activities. The temperature constants were observed by about 26 different physiologists.

One cannot compare these numbers with those of physical and chemical processes without being impressed with a few very remarkable facts:

1. The temperature velocities of the physiological activities are all of magnitudes similar to those of physical and chemical processes.
2. The coefficients for the most part and for those physiological activities where we know (because of other reasons) metabolism takes place, are of the magnitude of the coefficients for chemical reactions.
3. Those coefficients which are below the lower limit set for chemical action seem to simulate those of well-known physical actions.

The foregoing study has been made with a view toward indicating what value there may be in a comparison of the temperature velocities of physiological with those of physical phenomena. It was thought preferable first to exhaust the data already at hand in the literature before undertaking new experiments. It has been shown that this body of data suffices for the determination of the coefficients for certain activities, such, for example, as the rate of the heart beat, and the latent period of smooth and striated muscle contraction. On the other hand, the data for certain other activities is either incomplete or unreliable, and in some cases wanting altogether. Among these may be mentioned the influence of temperature upon the latent period of cardiac muscle, the latency of vagus stimulation, and the velocity of urine excretion and lymph formation.

## KIDNEY SECRETION OF INDIGO CARMINE, METHYLENE BLUE, AND SODIUM CARMINATE.

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IN 1842 Bowman<sup>2</sup> first published his theory of kidney secretion, in which he stated that the aqueous portion of the secretion is furnished by the Malpighian bodies and its "characteristic proximate principles" by the walls of the tubules. Only a little later (1844) Ludwig,<sup>3</sup> in opposition to this, advanced his "mechanical filtration" hypothesis. Then Heidenhain<sup>4</sup> (1874-5) attacked the problem from the standpoint of experimental physiology. He used color dyes and other bodies (as urea), and attempted to trace the passage of these through the kidney from the blood capillaries to the ureter. Chief among the dyes used were indigo carmine and ammonium carminate.

Heidenhain's experiments with indigo carmine are so generally known that certain of them need be described here only briefly.<sup>5</sup>

1. He reduced the blood pressure in a rabbit by section of the spinal cord, thereby cutting down the amount of water passed out by the glomerulus, and then injected into the blood only five c.c. of a saturated solution of indigo carmine. Within a few minutes the kidneys and the stain in them were fixed with absolute alcohol (indigo carmine being insoluble in absolute alcohol). Examination of these kidneys showed the stain in the cells of the convoluted tubules, and Heidenhain's statements lead one to infer — though he does not say so directly — that in this experiment no precipitate was found in the lumina of the convoluted tubules.

<sup>1</sup> I am glad to acknowledge my obligation to Professor KINGSBURY for help and suggestions in every part of the work. My sincere thanks are due to Dr. DRESBACH and Mr. PAWLING for help in the experiments; also to Professor GAGE for many kindnesses, and for the loan of apparatus from his laboratory.

<sup>2</sup> BOWMAN, W.: Proceedings of the Royal Society, London, Feb. 17, 1842.

<sup>3</sup> LUDWIG, C.: WAGNER's Handwörterbuch, ii, p. 637.

<sup>4</sup> HEIDENHAIN, R.: Archiv für mikroskopische Anatomie, 1874, x, p. 30. Archiv für die gesammte Physiologie, 1875, ix, p. 1.

<sup>5</sup> HEIDENHAIN, R.: HERMANN's Handbuch, 1881-3, v, pp. 345-351.

2. If a rabbit were treated in the same manner, and allowed to live for an hour before the kidneys were fixed, their examination showed blue granules of pigment in the lumina of the convoluted tubules and the wider limb of the connecting Henle's loop, but in no other places.

3. When larger amounts of indigo carmine were injected into the circulation of a rabbit, nuclei of the convoluted tubule cells became stained decidedly, and if the spinal cord were left intact, granules of blue precipitate appeared throughout the lumina of these tubules, Henle's loop and limbs, and in the collecting tubules where it had been washed by water from the glomerulus.

In no case did he find the cells of Bowman's capsule stained or any precipitate of the stain either in the lumen of the capsule or in that of the neck of the capsule.

4. Chrzonszczewski<sup>6</sup> and Wittich<sup>7</sup> had both shown that ammonium carminate, after injection into the blood, is passed out by the glomerulus. Heidenhain agreed with their results concerning that salt.

Heidenhain's interpretation of these results, and of many others, supported the Bowman hypothesis so strongly that it is now generally known as the Bowman-Heidenhain theory of kidney secretion. According to this view, water and inorganic salts in solution pass from the blood through the walls of the glomerulus, while most organic salts, the free acids, and a little water are secreted into the lumina of the proximal convoluted tubules, the wider ascending limbs of Henle's loops and the distal convoluted tubules by the lining epithelial cells of these tubules.

Strongly opposed to this view is the modified "filtration theory," which holds that although the glomerular capsule is able to exercise a selective action in keeping back entirely certain bodies (as serum albumen), still all the materials out of which urine is formed are passed through the capsule in its dilute glomerular filtrate. Then out of this dilute filtrate, as it passes along the lumina of the convoluted tubules and the wider limbs of the connecting Henle loop, their lining epithelial cells manufacture and concentrate the urine by the selective absorption of bases and water.

The two theories thus differ radically in the degree of selective activity attributed to the glomerular epithelium and in the function of the epithelium of the tubules mentioned.

<sup>6</sup> CHRZONSZCZEWSKI, N. : *Archiv für pathologische Anatomie und Physiologie*, 1864, xxxi, p. 187.

<sup>7</sup> WITTICH : *Archiv für mikroskopische Anatomie*, xi, p. 77.

Since Heidenhain's work was published, each theory has had many supporters bringing evidence from various sources. It is not to advantage here to review the experiments of all these workers, because the present paper is concerned especially with the secretion of *certain* dye stuffs — particularly indigo carmine and methylene blue — by the kidney.

Critical examination of the literature, however, shows no conclusive evidence as to how the kidney produces urine from the blood,<sup>8</sup> and often the results of very similar experiments in the hands of different workers have been contradictory. This is true of the work done with indigo carmine. Sobieranski's results<sup>9</sup> with this color body contradict those of Heidenhain in one essential point, and his interpretation is exactly opposed to that of Heidenhain.

Sobieranski deprived animals of water for many hours and gave them purging salts (Glauber's salt for example), by this means reducing the amount of water in the blood as much as possible. He then injected into the blood large amounts of indigo carmine, as much as 14 c.c. of the saturated solution to one kilo of body weight of a dog; and after varying lengths of time fixed the kidneys with absolute alcohol according to Heidenhain's method. He claims, in this way, to have obtained shortly after injection, blue-stained glomeruli. If the animal lived, after the injection, a somewhat longer time before the kidneys were fixed, then he found the cell-nuclei stained as Heidenhain had described, as well as the blue precipitate in the lumina of the convoluted tubules, Henle's loops, and the collecting tubules. The epithelial cells of a convoluted tubule, he says, showed the stain first and strongest on that side of the cells bordering the tubule. From these and similar results with sodium carminate, Sobieranski argues that the stain is passed out from the blood in a very dilute filtrate through the glomerulus, and that some of it becomes absorbed as it passes along the lumina of the tubules, thus staining their epithelial cells.

In view of these conflicting results it seemed worth while to profit by the work already done and to try further experiments with this same indigo carmine, with sodium carminate, and with methylene blue. Records were kept of the kidney secretion of these dyes in

<sup>8</sup> Cf. BEDDARD, A. P.: *HILL'S Recent Advances in Physiology and Biochemistry*, 1906—, conclusion, p. 727; also for bibliography.

<sup>9</sup> SOBIERANSKI, W.: *Archiv für experimentelle Pathologie, Pharmakologie, und Physiologie*, 1895, xxxv, p. 145.

eighteen cats and twenty-three rabbits. Either chloral hydrate or A.C.E. mixture was used as an anæsthetic for the cats, and for the rabbits pure ether was used.

In a large per cent of the experiments the spinal cord was divided in the region of the last cervical vertebra in order to reduce the general blood pressure and retard kidney secretion. The first injections were made with saturated solutions of indigo carmine — one gram to one hundred forty c.c. of water. The chemically pure indigo carmine powder was obtained from the Continental Chemical Co., New York City. Vorlander and Schubart<sup>10</sup> give the structural formula for this compound. It was obtained as a heavy blue powder, soluble in water to the extent mentioned above. From the water solution the compound may be precipitated almost completely by excess of absolute alcohol or by saturated salt solutions. The best results at fixing were obtained by the use of about one half per cent solution of picric acid in absolute alcohol. This not only precipitated the stain perfectly, but brought out cell structure much better than the alcohol alone. As a rule, one kidney was washed out through the renal artery by means of a large syringe with (50) fifty c.c. of the picric alcohol. It was then cut open and dropped at once into about the same amount of absolute alcohol. As a check, the other kidney was cut open, fresh, for macroscopic examination and then dropped directly into picric alcohol.

The following are some typical records of these experiments: Cat and rabbit experiments are numbered separately.

*Protocol. No. 5.* — Cat anæsthetized with "chloroform and ether" mixture. Injected 20 c.c. saturated indigo carmine, in period of 5 min., into femoral vein. Killed at the end of 15 min., and washed one kidney through the renal artery with absolute alcohol. Internal organs except liver, spleen and adrenals, were deep blue — kidneys especially blue. No blue in the urine. Under the microscope, cortex and outer part of the medulla of kidney decidedly blue — the precipitate in the lumen. Cells of the tubule showing little stain; their nuclei not stained. Blue color often showing on the inner walls of blood vessels of kidney. Bowman's capsule clear and glomeruli clear in the washed kidney.

*Protocol No. 10.* — Cat anæsthetized with chloroform and ether. Injected 10 c.c. of the saturated sol. of indigo carmine in 0.9 per

<sup>10</sup> VORLANDER and SCHUBART: *Berichte der deutschen chemischen Gesellschaft*, 1901, xxxiv, p. 1860.



cent salt solution (at blood temperature) into femoral vein. Injection period 10 min. Killed 2 hours later. Internal organs normal in color except the bladder which contained intensely blue urine. Cortex of the kidney clear. Only a slight amount of blue precipitate found in the larger collecting tubules of the medulla after fixing with absolute alcohol.

In no cat was more than twenty-five c.c. of the saturated indigo carmine solution injected. The cavity and the epithelial cells of Bowman's capsule never contained stain or precipitate. Glomeruli whose capillaries had been washed out with absolute alcohol were mostly entirely clear, while those that had been fixed by simply dropping the halved kidneys into absolute alcohol contained blue stain in their capillary tufts. Neither the cells of the convoluted tubules nor their nuclei ever took on more than a slight bluish stain.

Results of only the more typical rabbit experiments may be given here. The headings of the protocols give, in each case, the material used in the injection. In nearly all cases the injection was made into the femoral vein.

#### PROTOCOLS.

##### **Typical Injections of Saturated Solutions of Indigo Carmine. —**

*Protocol No. 2a.* — Kidney blood vessels tinged blue on their inner walls. Washed with alcohol through renal artery. Rabbit etherized until after the spinal cord was cut. Injection made in the femoral vein. Rate of injection 5 c.c. per min. Interval between beginning of injection and death 8 minutes. Amount injected, 30 c.c. Glomeruli nearly all clear; some faintly blue. Cells and cavity of Bowman's capsule clear. Proximal convoluted tubules — lumina with scattered precipitate; cells tinged faintly bluish green; nuclei in extreme outer cortex stained. Henle's loop and limbs — no loops show. Many limbs with blue precipitate. Distal convoluted tubules — lumina with scattered precipitate. Nuclei of cells tinged in places, blue. Collecting tubules — some filled and some nearly empty. Intestines blue. Adrenals without color. Urine slightly blue at the neck of the bladder.

*Protocol No. 8a.* — Rabbit etherized. Rate of injection 1.5 c.c. per min. Interval between beginning of injection and death 24 minutes. Amount injected — 33 c.c.m. Kidney washed out with 50 c.c.m. of picric alcohol. Glomeruli almost all clear. A few slightly tinged blue. Cells and cavity of Bowman's capsule clear. Proxi-

mal convoluted tubules — cells slight bluish green. Little precipitate in the lumina. Henle's loop and limbs — lumina with precipitate. Distal convoluted tubules — nuclei stained weakly except in outermost cortex. Little precipitate in lumina. Collecting tubules — lumina mostly filled with blue precipitate. Organs as above. A little picric alcohol washed into ureter.

**Injections of Saturated Solutions of Leuco-Indigo Carmine. —**

*Protocol No. 10 a.* — Rabbit etherized until spinal cord was cut. Rate of injection 25 c.cm. in 30 min. Interval between beginning of injection and death one hour. Amount injected — 25 c.cm. Kidney washed out and fixed with absolute alcohol. Glomeruli clear. Cells and cavity of Bowman's capsule clear. Proximal convoluted tubules — cells showing slight trace of greenish blue; faded somewhat in absolute alcohol. No precipitate in lumina. Henle's loop and limbs — no precipitate. Distal convoluted tubules — same as proximal tubules. No nuclei stained in either case. Collecting tubules — all filled with blue precipitate up through the boundary layer. Intestines, skin and "whites of eyes" remained natural color. Bladder filled with intensely blue urine.

*Protocol No. 12 a.* — Rabbit etherized throughout the experiment. Rate of injection 30 c.cm. in 20 min. Interval between beginning of injection and death 30 minutes. Amount injected — 30 c.cm. Kidney washed through the renal artery and fixed with picric alcohol. Glomeruli clear. Cells and cavity of Bowman's capsule clear. Proximal convoluted tubules — lumina with here and there scant ragged bits of blue precipitate. Inner faces of cells greenish blue. Henle's loop and limbs — no precipitate. Distal convoluted tubules — same as proximal part — the lumina here with a little more precipitate. Collecting tubules — precipitate in small collecting tubules very dense, growing less dense and scattered in large collecting tubules. Intestines, mouth and "whites of eyes" turned faintly greenish blue on death. Urine slightly blue.

**Typical Injections of Indigo Carmine in Suspension (and Solution) in Water. —**

*Protocol No. 15 a.* — Rabbit. Ether administration stopped as soon as injection needle was inserted. Cord not completely divided. Rate of injection 40 c.c. in 25 min. Interval between beginning of injection and death 30 minutes. Amount injected — 0.8 gm. suspended in 40 c.c. of distilled water containing 4 c.c. of hirudin. Kidney washed with picric alcohol and fixed. Glomeruli clear. Cells and cavity of Bowman's capsule clear. Proximal convoluted

tubules had flocculent blue precipitate in lumina. Nuclei faintly stained blue; cells weaker greenish blue. Henle's loop and limbs had much precipitate. Wider limb with cell nuclei stained faintly. Distal convoluted tubules — same as the proximal part. Collecting tubules — heavy precipitate in all collecting tubules. Urine blue. Skin and intestines very blue.

*Protocol No. 18 a.* — Rabbit. Etherized until after the cord was cut. Oxygen given. Rate of injection 50 c.c. in 25 min. Interval between beginning of injection and death 1 hour. Amount injected — 1.00 gm. in 50 c.c. of water with 4 c.c. of hirudin. After 10 min. a second injection of same amount (*i. e.*), 2 gm. in all in 100 c.c. water. Kidney washed out and fixed with picric alcohol. Glomeruli nearly all clear. A few showing slight stain in capillaries. Cells and cavity of Bowman's capsule clear. Proximal convoluted tubules — cells slightly greenish blue. Some nuclei stained. Lumina with much flocculent ragged precipitate. Henle's loop and limbs — lumen full of precipitate. Distal convoluted tubules — similar to proximal part. Collecting tubules — collecting tubules all packed with precipitate. Intensely blue urine in bladder, skin and intestines very blue. Medulla of adrenals plainly blue, but cortex clear. Gray matter of brain weakly but decidedly blue.

**A Typical Injection with Methylene Blue. —**

*Protocol No. 19 a.* — Rabbit etherized until cord was cut. Oxygen given. Rate of injection 15 c.c. in 12 minutes, — interval between beginning of injection and death; died at the end of 12 minutes. Amount injected — 15 c.c. of a 2 per cent solution. Kidney washed out and fixed with a 15 per cent solution of ammonium Molybdate in 1 per cent picric in absolute alcohol. Washed in water and hardened in absolute alcohol. Glomeruli clear. Cells and cavity of Bowman's capsule clear. Proximal convoluted tubules — no precipitate in the lumina. Cells stained a decided blue. Henle's loop and limbs — no precipitate. Cells of wider limb stained. Distal convoluted tubules — cells stained a decided blue. A very slight trace of precipitate found along the inner edge of cells in only a few places. Collecting tubules — no precipitate or stain. Urine natural color. Intestines and heart at first natural color but turned decidedly blue on death and exposure to air.

**Typical Injection of Sodium Carminate. —**

*Protocol No. 4 a.* — Rabbit etherized until the spinal cord was cut. Rate of injection 5 c.c. per min. Interval between beginning of

injection and death 25 minutes. Amount injected — 30 c.c. of a saturated solution. Kidney washed out through the renal artery and fixed in absolute alcohol. Cells of the epithelial lining of the glomerulus stained. Fine granules of precipitate in the Bowman's capsules. Scattered granules of precipitate in the lumina and along the walls of the proximal and distal convoluted tubules and limbs of Henle's loops. Collecting tubules — considerable amount of fine granular precipitate and some sticking along the inner walls of the lumina. Urine slightly red; bladder full. Intestine red.

Because of the manner of fixing, the unwashed "b" kidneys are always more shrunken than the washed "a" kidneys which, as a rule, are much the better fixed of the two. Still, the unwashed kidneys corresponding to the numbers given in the table show practically the same condition as their mate in every case. The glomerulus is of course always somewhat blue because the blood containing indigo carmine has not been washed out, but the Bowman's capsule in these kidneys shows no precipitate or stain; and the glomerulus itself is much weaker in color, as a rule, than the surrounding tubule tissue. In washing kidneys out through the renal artery with picric alcohol, a little of the yellow fixing fluid was observed always to pass into the ureter; but comparison with the corresponding unwashed kidney failed to show any material difference in the position of the precipitates in the lumina of the tubules.

The protocol of results of Experiments No. 1-9 inclusive, corresponds very closely with those of Heidenhain. His intimation, however, that he was able to obtain cells of the convoluted tubules stained without having any precipitate in the lumina of these tubules, — by injecting small amounts of the indigo carmine solution into the vein of a rabbit whose spinal cord was cut, and then killing soon after — was not verified. On the other hand if the cells of the convoluted tubules were stained at all in these experiments, there was always at least a little precipitate in the space of the convoluted tubules as well. Moreover, when the animal was killed in even ten minutes after the injection (the cord having been divided), small granules of precipitate were still found washed over into the smaller, outer collecting tubules. That is, the experiments seemed to indicate that Heidenhain laid too much stress on the ability of the divided cord to retard water secretion — but certainly, it does retard the secretion much.

Heidenhain's "corroding, or cauterization experiment" with

silver nitrate was not repeated because it hardly appeals as being trustworthy. From the structural relation of the capillary vessels of the kidney, the blood supply of the glomerulus could scarcely be affected without affecting that of the capillaries around the convoluted tubules in a corresponding area. But in any event, the results of Experiments No. 1-9 do favor strongly Heidenhain's interpretation; because the cells of the convoluted tubules were faintly stained, while those of Bowman's capsule or the cavity of this capsule never contained stain or precipitate. Yet absolute proof of this view is lacking in these experiments, since the staining of the tubule cells was always accompanied by some little precipitate in the lumina of the tubules, where it might have come from the capsule in a filtrate too dilute to give a noticeable precipitate until (according to Sobieranski) it had been concentrated by the absorptive action of the tubule cells, and they had been thus stained. Furthermore, Dreser<sup>11</sup> has shown that the glomerular filtrate is alkaline, and becomes acid as it passes along the lumina of the convoluted tubules. Now, it seemed not impossible that this alkaline filtrate might pass the indigo carmine as a reduced leuco-compound (indigo white?) which became oxidized to the blue indigo carmine as the filtrate became acid. Indeed, Sobieranski hinted at a reduction in the blood and in the glomerulus.

With this in mind, it was decided to make the leuco-compound, inject it into the circulation of a rabbit and note results. From test tube experiments it soon appeared that leuco-indigo carmine does not have the properties of indigo white. The gray crystals of indigo white are insoluble in water, and oxidize on contact with air to the original blue indigo compound. The steel-gray leuco-indigo carmine crystals, on the other hand, are as soluble in water as the indigo carmine itself. In the aqueous solution oxidation of this leuco-compound to indigo carmine occurs instantly in the presence of the least trace of oxygen. For this reason, it proved difficult to obtain a pure solution of the leuco-indigo carmine and introduce it as such into the rabbit's blood. This was finally accomplished with the apparatus shown in Fig. 1. A short description of this apparatus and the method may not be out of place:—

*C.g.* (Fig. 1) is a carbon dioxide generator; *w* a combined wash bottle and pressure regulator; *p* the tube of an adjustable "pressure bottle"; *m.f.*, a flask containing 2 gm. of indigo carmine, 3½ gm.

<sup>11</sup> DRESER, H.: *Zeitschrift für Biologie*, 1885, xxi, p. 41.

of zinc dust and  $\frac{3}{4}$  gm. of calcium chloride in 200 c.c. of distilled water; *F*, a filter funnel with the rubber connecting nipple clamped air-tight at the top; *g.t.*, a graduated containing-tube for the leuco-solution; and *n* is the small injecting needle. Tube *a* opens at the bottom of the wash bottle, a little below the opening of *p*. Then from the very top of the wash bottle *b* leads to the bottom of *m.f.*; and *c* connects the top of *m.f.* with the top of the rubber nipple.

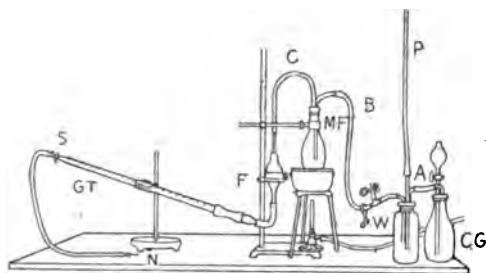


FIGURE 1.—Apparatus used in the manufacture, isolation, and injection, of leuco-indigo carmine.

The carbon dioxide generator was started and its stop-cock closed at the top. Carbon dioxide passed for an hour, until all air in the apparatus was certainly replaced. Then, with the carbon dioxide passing all the time, the contents of *m.f.* were heated over the water bath for an hour or more until the blue solution was thoroughly reduced to a yellowish straw-colored one. If at any time carbon dioxide was generated faster than it could escape (under moderate pressure) through the needle *n*, the water in *w* was forced up *p* until bubbles of carbon dioxide could escape through the pressure bottle. *M.f.* was now inverted. Carbon dioxide pressure (and gravity) forced the warm light straw-colored fluid through *c* to the filter. There all insoluble zinc hydroxide (from the reduction process) and unused particles of zinc dust were separated. This filtered leuco-indigo carmine solution then rose slowly in *g.t.*, pushing the "block" of carbon dioxide out through the needle before it until the whole tube and needle were filled with the solution. *M.f.* having been returned to its original position, the funnel-end of *g.t.* was carefully raised higher than the opposite needle end. The needle was dipped into a beaker containing water. The carbon dioxide pressure soon emptied the funnel, forcing the filtered solution through the needle until the carbon dioxide reached the first graduation mark of *g.t.* Then, stop-cock *s* was closed and the solution allowed to cool under pressure ready for the injection experiment.

This compound is more soluble in warm water than cold; so that on cooling, small crystals of the leuco-indigo carmine separated along the sides of *g.t.*, and thus a saturated solution was insured.

By keeping up the carbon dioxide pressure in the rubber tubes the air could be so effectually excluded as to keep the solution pure many hours and always ready for injection. Only a very little of the leuco-solution could oxidize at the tip of the small, immersed injection needle, if it were washed out by opening stop-cock *s*, for a moment just before the injection.

The results for two rabbits injected with leuco-indigo carmine in this way are given in protocol Nos. 10 a and 12 a. It may be seen here that the blood and tissues of the etherized rabbit were able to keep the leuco-compound in the reduced condition until death except in the lumina of the convoluted and collecting tubules and in the urine. Oxidation evidently took place in those parts of the lumina of the tubules which Dreser found to be acid. Not even by treatment with alcohol containing peroxide of hydrogen could the Bowman capsules be made to show any blue stain, although the cells of the convoluted tubules did appear a faint greenish blue. It had been previously determined by experiment in the test tube that alcohol would precipitate the leuco-indigo carmine, and that the precipitate would then turn blue in the presence of oxygen. The unwashed kidneys (10 b and 12 b), cut open immediately after death, showed already a deep blue medulla, a less deeply colored boundary layer, and a cortex almost clear.

Jaffe's test for *indikan* in the urine of these two rabbits and in that of several others injected with indigo carmine failed to show the presence of that body.

Here again the results, as in the former experiments, indicate that the injected compound was passed out of the blood through the epithelial cells of the convoluted tubules. Nevertheless it was decided to follow Sobieranski's suggestions further, and obtain if possible a more concentrated blood solution of indigo carmine. Fifty c.c. of a saturated (filtered) solution of this salt contains but little more than 0.35 gm. of the color compound, and this in the blood of a moderate-sized rabbit makes a solution so dilute as to afford a very scant precipitate indeed with absolute alcohol—a precipitate which might be overlooked in the dilute filtrate of the capsule. But Sobieranski's method of giving purging salts (also diuretics) and depriving the animal of water for many hours before the injection experiment, seemed hardly a safe one from which to draw conclusions. A more concentrated secretion might be obtained from the kidneys in that way, but certainly the method places those organs

under decidedly unusual (perhaps pathological) conditions before the real experiment is begun.

Accordingly, the purging salts were avoided, and after a number of trials it was found that water, containing as much as one gm. of dissolved and finely triturated indigo carmine suspended in 50 c.c., could be successfully passed through the injection needle. Attempts were made to inject the powder suspended in this way, with fatal results. Death appeared to be caused by some coagulation brought about by the fine particles of indigo carmine before they could be dissolved in the blood. Resort was therefore finally made to the use of hirudin<sup>12</sup> as a means of preventing this coagulation until the blood plasma could dissolve the fine particles of powder. Four c.c. of a solution of hirudin containing 0.1 gm. of that body in 25 c.c. of a 0.6 per cent salt solution were found sufficient. As a second precaution arrangement was made to give the animal oxygen whenever necessary throughout the experiment. Injections of large quantities of the color solution were best accomplished by means of a graduated containing tube, for the solution, connected above with an air chamber whose pressure was kept at any desired constant by means of a suspended water-pressure bottle.

Results of two experiments in which finely suspended indigo carmine was injected by the method described, are given in the protocols under Nos. 15 a and 18 a. The record under 18 a is that of the largest amount (2 gm.) of indigo carmine injected in a rabbit. The heart action was strong and regular at the end of the experiment before the rabbit was killed, and he was able to perform his own respirations. The greatest amount of saturated solution injected by Sobieranski in any experiment is equivalent to only 0.36 gm. of indigo carmine per kilo of body weight.

Still, in all these "powder suspension" experiments the Bowman's capsule remained free from stain and precipitate. Blue granular and flocculent precipitate was found, however, in quantity in the places already described for the earlier experiments, and as given in the protocols.

Dreser in his paper ('85) says that methylene blue is excreted by

<sup>12</sup> Hirudin used in these experiments was obtained in sealed glass tubes from Bischoff & Co., 88 Park Place, New York. For physiological action of hirudin see HAYCRAFT: *Archiv für experimentelle Pathologie und Pharmakologie*, 1884, xviii, p. 209, and KAPOSI: *Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie*, xiii, No. 3.



the kidney in the reduced or leuco-condition, and he believed that it was passed out by the cells of the convoluted tubules, through whose agency the reduction was accomplished.

Ehrlich<sup>13</sup> ('85) and several workers more recently<sup>14</sup> have studied the reducing action of different tissues of the animal body on methylene blue under various conditions, and its oxidation on exposure to air after death. Underhill and Closson<sup>15</sup> ('05) have called attention to Bernthsen's statements concerning the chemistry of this reduction. In alkaline solution methylene blue (salt) first goes to methylene blue (base), then on reduction to leuco-methylene blue (base).

On looking up and testing the properties of this leuco-methylene blue, the compound was found to be but *very slightly* soluble in water, while the methylene blue itself is much more soluble, even, than indigo carmine. Moreover, leuco-methylene blue oxidizes very much more slowly than leuco-indigo carmine; so that one may watch the process of change of color gradually take place. These properties seemed to offer decided advantages if only the compound could be precipitated and fixed well in the tissues. It appeared that as soon as the cells reduced the methylene blue, it would at once be in a condition almost insoluble, and the process of excretion would necessarily be slow. Time would therefore be afforded after injection to kill the animal, and to oxidize and fix the compound in the tissues that were reducing it, before they had time to complicate matters by secreting it into the kidney canals. This hypothesis proved to be correct. The process of excreting was so slow that even at the end of a half hour from the time of injection of the methylene blue into the blood, not enough of the material had passed into the lumina of the convoluted tubules to reach the collecting tubules of the medulla. That is, the medulla remained natural color after contact with the air or hydrogen peroxide, while the cortex turned blue. In the experiments with methylene blue one kidney was washed out through the renal artery with 50 c.c. of a 15 per cent solution of ammonium molybdate containing 1 per cent picric acid and a little peroxide of hydrogen. This oxidized the leuco-compound

<sup>13</sup> EHRLICH, P.: *Centralblatt für die medicinischen Wissenschaften*, 1885, xxiii, p. 113.

<sup>14</sup> Two of these are ELSNER: *Deutsches Archiv für klinische Medizin*, 1901, lxi, p. 47, and HERTER, C. A.: *This journal*, 1904, xii, pp. 128 and 207.

<sup>15</sup> UNDERHILL, F. P. and O. E. CLOSSON: *This journal*, 1905, xiii, p. 358.

and fixed a blue precipitate insoluble in alcohol or water. After treatment with this fixing fluid, the kidney was washed in water, to remove the excess of ammonium molybdate, and then hardened in alcohol. No. 19 a in the protocols gives the showing of a microscopic examination of such an experiment with methylene blue. The Bowman's capsules remained without color. The cells of the distal and proximal collecting tubules and the larger limbs of Henle's loops were stained a decided blue and there was no noticeable precipitate in the lumen. The conclusion here is almost irresistible that the cells had received the methylene blue compound from the blood and had not absorbed it from the lumina of the tubules since these little canals were without any color precipitate.

The other kidney of the rabbit was cut into halves without being washed. The cut surfaces were at first natural color, but after a few minutes' contact with air one could observe the cortex turn blue from the oxidizing leuco-compound. The medulla remained *natural color*. That the color change in the cortex did not come from stain in the cut blood vessels is clear when one remembers that the medulla also is richly supplied with blood vessels. Half of this kidney was now fixed for eight hours in ammonium molybdate, picric acid, peroxide of hydrogen mixture. It was then washed two hours in water, hardened in alcohol and sectioned. These sections afforded the same results as did the other kidney.

As noted under remarks in the protocol (19 a), the tissue of the intestine and heart were found to contain the leuco-methylene blue which oxidized and so turned the tissues blue upon death and exposure to air. One could cut across the fresh heart muscles and then watch the blue color appear and intensify on the cut surface, just as in the case of the cortex of the kidney.

Pieces of the small intestine were fixed in the ammonium molybdate-picric acid mixture. They were then washed in water, hardened in alcohol and sectioned. These sections showed the stain to be principally in the mucosa — and as Professor Gage kindly pointed out to me, the goblet cells are the most deeply stained.

As a rule, 12 to 15 c.c. of a 2 per cent solution of Merck's highest purity medicinal methylene blue injected into the femoral vein of a rabbit would prove fatal after a few minutes — at most one half hour.

One rabbit was allowed to swallow a capsule containing 0.4 gm. of methylene blue, and two hours later a second capsule containing

a like quantity was given. The rabbit was then killed and examined four and one-half hours after the first dose. In this time the doses had caused no bad effects. The bladder contained about 8 c.c. of very blue urine. The food of the stomach and small intestines still held a great deal of the methylene blue which could be dissolved out with water. The mucosa of the stomach and intestines were blue wherever the blue food was in contact with it. One kidney was fixed in ammonium molybdate and picric acid. Microscopic sections showed no noticeable blue color in the cortex and only slight color in the boundary layer, but the medulla was decidedly blue. Examination with the microscope showed this stain to be along the walls of the collecting tubule lumina in the medulla. The cells of the larger limb of Henle's loop and those of the convoluted tubules in the outer cortex showed a faint tinge of blue under the low power of the microscope.

The other kidney was cut open in the fresh condition. It was at first without blue color, but with a few minutes' exposure to the air the medulla alone turned quite blue. Evidently the methylene blue had been passed from the kidney as *leuco-methylene* blue (at least as leuco-compounds — perhaps leuco-methylene azure is also present) which had been oxidized to the blue compounds again in the bladder.

Nuclei of cells of the convoluted tubules were in no case noticeably stained with methylene blue as was so often found to be true when indigo carmine was used. The intensity of the nuclear stain with this latter color body, however, seemed to vary in different rabbits, in some measure, independent of the injection time and the quantity of color material used. Furthermore, in the experiments in which leuco-indigo carmine was injected, no nuclei of the convoluted tubule cells were stained, and the cells only faintly so. This result with the leuco-compound accords with Lillie's work<sup>16</sup> ('02) on the oxidation properties of the cell nucleus. He found that although the nucleus is the centre for synthetic processes in the cell and its chief seat of oxidation, still the cell body of the tubule cells of the kidney stained diffusely while their nuclei remained comparatively clear in the presence of "oxidation indicators" (as for example the Indophenol reaction).

Mathews<sup>17</sup> ('98) has shown that the "acid stains," in the pres-

<sup>16</sup> LILLIE, R. S.: This journal, 1902, vii, p. 412.

<sup>17</sup> MATHEWS, A.: This journal, 1898, i, p. 445.

ence of a little free acid, stain albumins or albuminoses while in neutral reaction the latter would take no color. Now, according to Ehrlich<sup>18</sup> ('79), indigo carmine is an acid stain, being the sodium salt of the disulphonic acid of the indigo color material. It is well known, also, that the amount of free acid of the urine varies, — being at times practically nothing, — and that the appearance of free hippuric acid, for instance, is due to synthetic processes in the kidney. Indigo carmine, then, should give blue stain with the nucleoproteids of the tubule cells when these are acid, and the intensity of the stain should vary with their acidity. This explanation of the staining of the tubule-cell nuclei by indigo carmine, and their freedom from stain after injection of leuco-indigo carmine into the blood, would necessarily indicate that the blue compound is not reduced in the cells of the kidney — at least not completely so when even moderate amounts are injected. Dreser in his work ('85) states that indigo carmine is free from reduction in the kidney.

Sobieranski obtained from the blood of an animal injected with indigo carmine, a bluish green serum by means of a centrifuge. His statement that this blue-green, instead of an indigo-blue color, is "conditioned by the strong reducing power of the living animal body" is not proof. An indigo carmine water solution of equal dilution appears blue-green also — even its scant precipitate as well.

It would seem at first that methylene blue, having been reduced by the cytoplasm of the tubule-cells, might be oxidized at the "synthetic centre of the cell" and so stain the nucleus. Underhill and Closson have shown, however, by Bernthsen's statements, that if leuco-methylene blue (base) is made slightly acid, a new compound results which is less easily oxidized than the leuco-compound (base). This new compound is a leuco-methylene blue (salt). Oxidation would therefore actually be hindered from taking place in the neighborhood of the nucleus during the life of the convoluted tubule cell. At least, if it did occur, (according to Underhill and Closson) leuco-methylene azure would result. No blue color would appear; and certainly, it is true, as has already been pointed out in the results of these experiments, that the nuclei of the tubule cells are not noticeably stained after injection with methylene blue.

Several injections of 20 to 30 c.c. of saturated sodium carminate solution were tried with cats and rabbits. Sobieranski has rightly

<sup>18</sup> EHRLICH, P.: *Archiv für Physiologie*, 1879, p. 571.

pointed out sodium carminate as more desirable for injection experiments than the ammonium salt, since the latter in solution is apt to yield some free ammonia. The chemical formula of sodium carminate is  $\text{Na}_2\text{C}_{17}\text{H}_{16}\text{O}_{10}$ . There seems to be not even a suspicion that this compound meets with any reduction in the animal body.

The results of a typical experiment with injected sodium carminate are given in the protocol No. 4 a. The epithelial lining of the glomerular capsule was found to be always deeply stained, and particles of precipitate were thrown down in the capsule and lumina of the tubules by the use of absolute alcohol.

Finally, the results of these experiments in the main, support the Heidenhain view of kidney secretion, because they show that:—

1. Almost (perhaps not entirely) without doubt, indigo carmine, when present in the blood even in large amounts, is passed into the urine through the cells of the distal convoluted tubules, the proximal convoluted tubules, and the wider limbs of Henle's loops.

2. Leuco-indigo carmine, injected into the circulation, may be held as such in the blood and tissues of the living rabbit. It is oxidized immediately after passing into the lumina of the convoluted tubules, where it comes, as the facts of the experiments indicate strongly, as a secretion from the cells of these tubules.

3. Methylene blue, shortly after being introduced into the blood, is found (in the kidney) as a reduced colorless compound only in the cells of the proximal and distal convoluted tubules, as also in those of the wider limb of Henle's loop. The reduced leuco-compound is then slowly secreted by these cells into the kidney tubules, from which it passes with the urine to become oxidized to the blue compound (in large part at least) in the bladder.

4. There is no direct proof that indigo carmine is reduced in the living animal body; and certain facts show that it is at least not completely reduced in the blood or in the tissues of the kidney when injected even in moderate amounts.

5. Sodium carminate, injected into the circulation, passed from the blood through the walls of the glomerulus into the cavity of Bowman's capsule just as has been generally agreed by all other workers.

It should be added here, however, that if the animal is allowed to live for fifty minutes to an hour after a large injection of the sodium carminate solution, the cells of the convoluted tubules show a faint reddish stain. This might be due to an absorption of the stain from the lumina of the tubules (as Sobieranski believed), or to a *slower*

*secretion* of the color body by the cells named — one cannot tell. But this much seems certain — that most of the sodium carminate, at least, is passed from the blood through the glomerulus while the methylene blue, on the other hand, is excreted by the cells of the tubules as already explained.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE  
HEART. — X. A NOTE ON THE PHYSIOLOGY OF  
THE PULSATING BLOOD VESSELS IN THE WORMS.

By A. J. CARLSON.

[From the Marine Biological Laboratory, and the Hull Physiological Laboratory of the  
University of Chicago.]

THE following observations were made at Woods Hole during the summer of 1907. The writer is not in position to continue and complete the work at present. The excuse for reporting the results in the present incomplete condition is the hope that they might induce biologists having the advantage of marine fauna to extend the observations, as the marine worms are best suited for the work.

I. The nervous tissues in the pulsating vessels of *neries* and *arenicola*.

In *arenicola* the œsophageal hearts as well as the dorsal or super-intestinal vessel may be completely isolated from adjacent tissues. This is not easily accomplished in *neries*, especially in the case of the dorsal vessels. When these isolated vessels in their living condition are treated with a dilute solution of methylene blue in sea water the nervous tissues in the walls of the blood vessels are stained blue in favorable preparations, while the muscle cells remain unstained. The types of nerve cells found in the vessel walls of *neries* and *arenicola* are shown in Figs. 1 to 3. The cells are mostly unipolar and bipolar. Occasionally a multipolar cell is met with. The axis cylinder processes and nerve fibres usually exhibit the varicosities typical of non-medullated fibres in *intra vitam* methylene blue staining. As far as could be determined, the nerve cells are not massed in definite regions or centres, but scattered throughout the whole of the vessel wall. In *arenicola* the nervous tissue is more readily brought out methylene blue in the dorsal blood vessel than

in the œsophageal hearts. In fact, I succeeded in obtaining what appears to be undoubted nerve cells in only two preparations of the œsophageal hearts of this worm, although many attempts were made.

How can we be sure that the elements just described and figured are nervous tissues? How do we know that they are not connective tissue cells and fibres? The reasons for calling these tissues nervous are: (1) structure, (2) staining reactions, and (3) anatomical con-

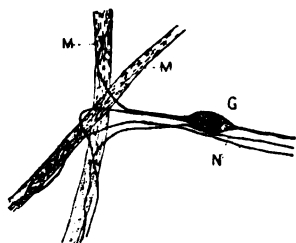


FIGURE 1.—Camera drawing. *a*, ganglion cells and portion of the nerve plexus in the walls of the dorsal blood vessel of nematode; *g*, ganglion cell; *m*, strands of muscle cells; *n*, nerve fibres. From a methylene blue preparation.

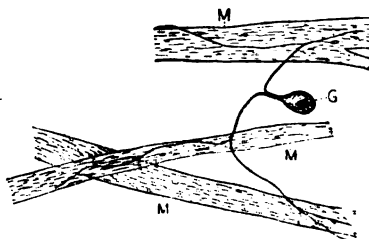


FIGURE 2.—Camera drawing. *a*, ganglion cell and its processes ramifying on the muscle strands in the œsophageal heart of nematode; *g*, ganglion cell; *m*, muscle strand. From a methylene blue preparation.

nections. The forms of the cells appear identical with some of the unipolar and bipolar cells in the ventral ganglia. The varicosities of the cell processes are those typical of non-medullated nerve fibres in the vertebrates including the worm. Such varicosities are not known to appear in connective tissue fibres under methylene blue staining. And finally, some of the axis cylinders can be traced into the nerve plexus that surrounds the muscle strands.

Obviously, then, the pulsating blood vessels of the worms possess the tissues invariably associated in the heart of vertebrate and invertebrate, *viz.*, muscle cells, ganglion cells, and nerve fibres.

II. The influence of the brain and the ventral nerve cord on the pulsating vessels.

(1) Attempts to establish nervous connections between the pulsating vessels and the nerve cord by macro- and microscopic methods gave negative results. As far as the writer is aware, nervous connections between the central nervous system and the blood vessels have never been described, possibly never even investigated.



(2) In arenicola stimulation of the ventral nerve cord with the weak interrupted current usually inhibits the œsophageal hearts in diastole while the rate and strength of the pulsations of the dorsal vessel are augmented. The stimulation never inhibits the dorsal vessel. A further indication of the presence of cardio-regulative nerves in the worms is the fact that in neries the pulsations of the dorsal vessel in the intact animal are much more variable than after the extirpation of the ventral nerve cord. These experiments do not prove the point, however, because on the stimulation of the nerve cord contractions are induced in structures adjacent to the pulsating vessels, and we have no means of proving to what extent these contractions influence the rhythm.

That the nerve plexus in the pulsating blood vessels is connected with the central nervous system is probable on *a priori* grounds, as we know of no case in the animal kingdom of a peripheral nerve plexus of this type isolated from the central nervous system.

(3) Tension on the vessel wall and hydrostatic pressure in the heart cavity appear to act in the same way as in the molluscan, the anthropod, and the vertebrate heart.

III. Some physiological properties of the blood vessel tissues:—

The isolated dorsal vessels and œsophageal hearts of neries and arenicola continue in rhythmic activity, hence all the mechanisms for imitating and maintaining the rhythm are to be sought in the vessels themselves, whatever be the connection of the vessel with the central nervous system.

The œsophageal hearts of arenicola exhibit a typical systolic refractory state, or condition of diminished excitability; but strong stimuli produce contraction at whatever phase of the beat they are sent through the heart.

The pulsating vessels exhibit the same tendency as the body musculature to go into prolonged and extreme tonus on strong stimulation. This tonus contraction cannot be distinguished from tetanus. On direct stimulation of the dorsal vessel this type of contraction is induced only in the part immediately stimulated. In the more

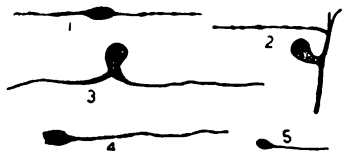


FIGURE 3.—Types of ganglion cells in the walls of the superintestinal blood vessel of arenicola. Methylene blue preparation. The processes of the cells 1 and 4 could be followed for a considerable distance in the longitudinal direction of the vessel wall.

distant part of the vessel the only effect of the stimulation is an augmentation of the rhythm. Whatever be the mechanism of conduction of the contraction wave in the blood vessels of the worms it is obvious that this extreme tonus or tetanus is not conducted.

The dorsal blood vessel and the œsophageal hearts are polarized so as to beat in one direction only. The mechanism of this polarization is not known. It is probably located in the pulsating vessel itself although it is difficult to demonstrate the persistence of this normal polarization in the isolated vessels. The vessels are capable of conducting the contraction wave in either direction, and the normal direction may be reversed by producing an extra contraction anteriorly.

A contraction wave once started in the posterior end of the dorsal vessel in the intact worm does not always traverse the whole length of the vessels. It may, and often does, fade away in the middle region of the heart. Contraction waves may start in the middle region in the intact animal. This variability in the conduction of the contraction wave reminds one of the peristalsis of the vertebrate intestines, and suggests a complex coördinating nervous mechanism, such as has now been proven for the arthropods (by Carlson) and for the reptiles (by Kronecker and Imchanitzky).

IV. Some of the echinoderms have a distinct vascular system developed in association with the alimentary canal. This vascular system has a musculature of its own, and, at least in some species, appears to be more or less rhythmically active. In the worm phylum the vascular system reaches a considerable development. And here at the very threshold of the heart rhythm, speaking phylogenetically, we are confronted with essentially the same fundamental conditions as in the heart of the highest vertebrate. There are the same tissues, viz., muscle, ganglion cells, and nerve plexus; and apparently the same type of regulatory nerves: motor and inhibitory. Essentially the same problems of the initiation and conduction of the contraction, of the properties of the automatic and conducting tissues, of the coördination of the rhythm, etc., confront us in the heart of the earthworm as in the heart of the dog.

# RESECTION AND END-TO-END ANASTOMOSIS OF THE OVIDUCT IN THE HEN, WITHOUT LOSS OF FUNCTION.

BY RAYMOND PEARL AND FRANK M. SURFACE.

[*Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 5.*]

IN this laboratory a series of investigations are under way regarding the physiology of the process of egg production in the domestic fowl. In connection with this work an attempt is being made to gain more complete and definite information than now exists concerning the functions and normal physiological activity of the different portions of the oviduct in the hen. One line of investigation which is being prosecuted towards this end involves the experimental removal of portions of the oviduct and a study of the resulting effects on the processes involved in the formation and laying of the egg. It is believed that in this field of the physiology of reproduction significant and valuable results may be obtained by the application of experimental surgical methods. The work of Pawlow and his followers in the application of such methods to the study of the physiology of digestion has demonstrated what the value to the physiologist of an adequate surgical technique may be.

The purpose of the operation which forms the subject matter of the present paper was to determine, as a necessary preliminary to further work, whether it was possible to remove a portion of the oviduct in a laying hen and to reunite the two cut ends without permanent loss of the function of egg production. The oviduct in birds is a particularly complicated and delicately balanced organ. In the greater portion of its length it is highly glandular. During the period of laying activity of a bird the glandular portion (albumen secreting and shell secreting glands) becomes very much enlarged and has its walls greatly thickened. In the laying hen the oviduct in the albumen secreting portion has a thickness of from 2 to 4 mm. The glandular portion of the oviduct is furthermore

highly vascular during the period of activity. In view of these facts the outlook for obtaining a successful anastomosis of this organ did not appear particularly hopeful at the outstart. Experiments on the matter were begun during the past winter, and carried on until the supply of suitable material was exhausted. The results were from the beginning more successful than had been anticipated. It is the purpose of this paper to set forth the remote results of one of our cases. This case shows that it is possible to remove a relatively large piece of even so highly glandular an organ as the laying hen's oviduct and by proper methods to obtain a perfectly functional end-to-end anastomosis.

#### OPERATION.

The bird used for this operation was a pure bred Barred Plymouth Rock pullet hatched in the spring of 1907. She began laying early in the winter of 1907. In January, 1908, her egg record was as follows: an egg was laid on January 3d, 4th, 6th, 8th, 9th, and 12th. On the 14th of January, 1908, the bird was isolated. On January 16th the operation for anastomosis of the oviduct was performed. Under ether anæsthesia<sup>1</sup> a piece approximately 10 cm. in length was removed from about the middle of the albumen secreting portion of the oviduct. Before removal the blood supply to the resected portion was tied off with fine silk ligatures. Bleeding from the cut ends of the oviduct was controlled by broad tape ligatures (not too tightly tied) about a half centimetre back from each cut end.

Anastomosis was made by a method fundamentally similar to that used by Carrel<sup>2</sup> for the anastomosis of blood vessels. Three retention sutures of No. 1 China silk were inserted and tied at equidistant intervals around the periphery of the ends to be anastomosed. These retention sutures involved all the layers of the oviduct wall. The ends of these sutures were left long. Traction was made on the ends of two of these retention sutures by the operator and his assistant. Then the two cut edges between the retention sutures were drawn together and turned in by a continuous Lembert suture made with one strand of fine silk. This silk was obtained by un-

<sup>1</sup> By a method to be described in a subsequent paper.

<sup>2</sup> CARREL, A.: Johns Hopkins Hospital Bulletin, 1907, xviii, No. 190, January, and other papers.

twisting Pearsoll's No. 0000 silk. In passing it may be said that this silk has been found to be excellent as a fine suture material. It is unusually strong. The other two sides of the triangle included by the retention sutures were approximated in the same way by continuous Lembert sutures. The body wall was closed in three layers. The recovery from the anæsthesia was slow but good. The wound

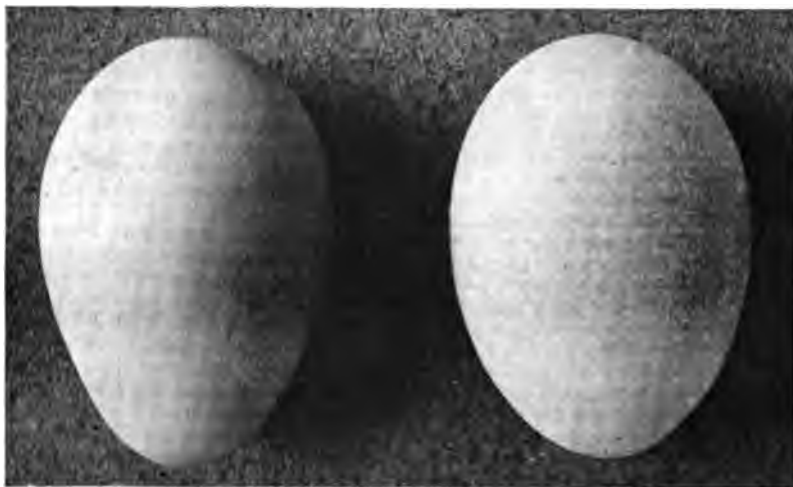


FIGURE 1. — Photographs (actual size) of the first two eggs laid by the hen described in the text.

was dusted with iodoform and covered with flexible collodion. Healing was by first intention, and a week after the operation the bird was back in one of the experimental pens with other birds; marked, of course, with a distinguishing leg band.

#### RESULTS.

Owing to a lack of proper housing facilities this bird was kept during the remainder of the winter and spring in a house which from previous experience was known to be unsuited for laying hens. The behavior of the hen early in March showed, however, that the normal instinct for egg production at this period of the year was asserting itself. The bird went frequently on the nest and in other ways gave evidence of being about to begin laying. On May 17, 1908, she actually did begin to lay again with the production of the

egg shown at the left in Fig. 1. On the following day, May 18, she laid the second egg shown in Fig. 1. Since that date she has continued to lay with regularity. It may thus be taken to be demonstrated that resection of a portion of the active oviduct and subsequent anastomosis may be made without any loss of function. The suggestiveness of this result for operative work on the Fallopian tubes of mammals is clear. Considering the histological structure of the Fallopian tube as compared with the actively functioning oviduct of a bird it would seem that the operation ought to be much easier and more uniformly successful in mammals than in birds.

A brief statement regarding the eggs laid by this hen after the operation is desirable. The dimensions of the first four eggs are given in the following table.

	Date laid.	Weight in gm.	Length in mm.	Breadth in mm.
First egg . . . .	May 17, 1908	47.0	56.7	39.0
Second egg . . . .	May 18, 1908	49.0	55.8	39.6
Third egg . . . .	May 19, 1908	46.9	53.8	38.6
Fourth egg . . . .	May 21, 1908	48.8	55.0	40.1

From these figures and the photographs it will be seen that the eggs are not widely divergent from normal size.<sup>8</sup> In a statistical study of the dimensions of the Barred Plymouth Rock egg which is now in progress in this laboratory constants for the normal size of the egg have been determined, and it may be stated that these eggs are but slightly below the average for the breed. The shell in all of these eggs was entirely normal in thickness and texture. In shape the first egg laid was slightly abnormal. It will be seen from the photograph that it was somewhat more pointed at the smaller end than is a perfectly normal egg such as the second laid by this same bird. The eggs laid on May 18 and later dates were normal in

<sup>8</sup> Measurements have been made of all the eggs laid by this hen, but since they exhibit only the slight variation normally found in the size of eggs laid by the same individual, there seems to be no reason for publishing detailed data beyond those given in the table.

shape. An examination of the contents of the eggs showed them to be entirely normal. There was no inclusion of blood in the white of any of the eggs. The relative proportion of albumen to yolk was substantially that of a normal egg. The chalazæ of the eggs were normal.

SUMMARY.

It is shown in this paper that:

(1) A piece of the albumen secreting portion of the actively functioning oviduct in the hen may be resected and an end-to-end anastomosis be made without permanent loss of function.

(2) The eggs produced by a hen on which this operation has been performed are normal, except for a slightly smaller size than the average for normal hens of the same breed.

## HYDROLYSIS OF VIGNIN OF THE COW-PEA (VIGNA SINENSIS).<sup>1</sup>

By THOMAS B. OSBORNE AND FREDERICK W. HEYL.

[From the Laboratory of the Connecticut Agricultural Experiment Station.]

A STUDY of the seeds<sup>2</sup> of the cow-pea showed that the principal protein constituent was a globulin, freely soluble in 5 per cent sodium chloride solution and nearly insoluble in a 1 per cent solution of this salt. As extensive fractional precipitations of this globulin gave a number of different preparations of constant composition and properties which showed no indication of a mixture of different proteins, and as this globulin was distinct from any previously described, it was named Vignin.

The vignin used for this hydrolysis was made by extracting the ground cow-peas with 5 per cent sodium chloride solution, filtering the extract perfectly clear, and dialyzing it for three days.

The precipitate produced by dialysis was filtered out, suspended in a measured quantity of water, and dissolved by adding a weighed amount of sodium chloride. The solution was then filtered perfectly clear and the vignin precipitated by diluting with water until the solution contained 1 per cent of sodium chloride.

The precipitate was allowed to settle, the supernatant solution drawn off, and the precipitate sucked as dry as possible with the pump. After washing with alcohol and ether the preparation formed a pure white, fine dusty powder. By this method of preparation, the vignin was separated from all the water soluble constituents of the seed as well as from more soluble globulins, albumin, and proteose.

### HYDROLYSIS OF VIGNIN.

Of the vignin, 511 gm., ash and moisture free, were dissolved in 1200 c.c. of hydrochloric acid, sp. gr. 1.1, by heating on a water

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

<sup>2</sup> OSBORNE and CAMPBELL: *Journal of the American Chemical Society*, 1897, xix, p. 494.



bath for three hours. The solution was then boiled in an oil bath for nineteen hours. After concentrating the solution and saturating it with hydrochloric acid gas, it was kept on ice for several days in order to separate glutaminic acid hydrochloride. It was, however, found to be impossible to bring this substance to separate in a condition in which it could be filtered from the solution. After several further unsuccessful attempts, the removal of the glutaminic acid was abandoned and esterification of all the amino-acids was conducted in the usual manner. The esters were shaken out with ether, the salts removed from the aqueous layer, and the esterification of the remaining amino-acids was repeated as before. The ether was distilled from the esters on a boiling water bath and the esters distilled, under diminished pressure, with the following results.

Fraction.	Temp. of bath up to.	Pressure.	Weight.
I	72°	17.00 mm.	20.69 gm.
II	100°	10.00 "	43.62 "
III {	a 93°	0.69 "	37.67 "
	b 100°	0.70 "	51.71 "
	c 106°	0.55 "	21.21 "
IV	126°	0.50 "	48.71 "
V	148°	0.48 "	53.20 "
VI	196°	0.45 "	47.45 "
Total . . . . .			324.26 gm.

The undistilled residue weighed 45 gm.

*Fraction I.* — This was evaporated on the water bath with an excess of hydrochloric acid and found to consist mostly of alcohol and ether. The residue was esterified, but no glyccoll ester hydrochloride could be obtained. We were further unable to obtain glyccoll from the ether distilled from the esters. The free amino-acids were regenerated and the solution evaporated to dryness. The residue weighed 2.95 gm., and examination showed it to be a mixture that could not be separated into products of definite character.

*Fraction II.* — This fraction was saponified by boiling with water, with reflux condenser, for nine hours, when it became neutral to litmus.

After evaporating to dryness, proline was removed in the usual manner by extracting with alcohol.

The amino-acids insoluble in alcohol were fractionally crystal-

lized and 10.27 gm. of leucine, 1.75 gm. of valine, and 5 gm. of alanine were obtained. Glycocoll was not present.

The valine when dissolved in 20 per cent HCl showed a specific rotation of  $(\alpha)_{D}^{20} = +27.5^{\circ}$ , and gave the following analysis:

*Carbon and hydrogen*, 0.1523 gm. subst., dried at  $110^{\circ}$ , gave 0.2875 gm.  $\text{CO}_2$  and 0.1308 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_6\text{H}_{11}\text{O}_2\text{N} = \text{C } 51.28$ ;  $\text{H } 9.40$  per cent.

Found . . . =  $\text{C } 51.48$ ;  $\text{H } 9.54$  " "

The alanine decomposed at  $285^{\circ}$ – $287^{\circ}$ , and gave the following analytical results:

*Carbon and hydrogen*, I. 0.1336 gm. subst., gave 0.1973 gm.  $\text{CO}_2$  and 0.0960 gm.  $\text{H}_2\text{O}$ . II. 0.1670 gm. subst. gave 0.2461 gm.  $\text{CO}_2$  and 0.1184 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_3\text{H}_7\text{O}_2\text{N} = \text{C } 40.45$ ;  $\text{H } 7.87$  per cent.

Found . . . { I. =  $\text{C } 40.27$ ;  $\text{H } 7.97$  " "  
                  } II. =  $\text{C } 40.19$ ;  $\text{H } 7.87$  " "

*Fraction III, a and b.* — This fraction was saponified by boiling with water for nine hours until neutral to litmus, the solution evaporated to dryness, and proline extracted from the dry amino-acids with alcohol. From the part insoluble in alcohol, 29.72 gm. of leucine were obtained.

*Carbon and hydrogen*, 0.1483 gm. subst., dried at  $110^{\circ}$ , gave 0.2996 gm.  $\text{CO}_2$  and 0.1358 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_6\text{H}_{10}\text{O}_2\text{N} = \text{C } 54.96$ ;  $\text{H } 9.92$  per cent.

Found . . . =  $\text{C } 55.09$ ;  $\text{H } 10.18$  " "

The proline was converted into its copper salt, and weighed as such.

The amount found was equivalent to 26.82 gm. of proline, or 5.25 per cent. It was identified as the phenyl-hydantoin of lævo-proline, which crystallized from a large volume of water in prisms which melted at  $143^{\circ}$ , and gave the following analysis:

*Carbon and hydrogen*, 0.1559 gm. subst., gave 0.3815 gm.  $\text{CO}_2$  and 0.0771 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_{12}\text{H}_{12}\text{O}_2\text{N}_2 = \text{C } 66.67$ ;  $\text{H } 5.57$  per cent.

Found . . . =  $\text{C } 66.73$ ;  $\text{H } 5.49$  " "

*Fraction III c.* — The phenylalanine ethyl ester was removed with ether in the usual way, and converted into the hydrochloride,

which weighed 3.84 gm. The free phenylalanine decomposed at about 274°, and gave the following analysis:

*Carbon and hydrogen*, 0.1656 gm. subst., gave 0.3991 gm. CO<sub>2</sub> and 0.1003 gm. H<sub>2</sub>O.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>N = C 65.45; H 6.66 per cent.

Found . . . . = C 65.72; H 6.73 " "

The aqueous layer was saponified by warming for eight hours with barium hydroxide, and 4.52 gm. of aspartic acid was obtained as the barium salt, 1.68 gm. of glutaminic acid hydrochloride, and 5.65 gm. of copper aspartate, which separated in the characteristic sheaves.

The aspartic acid reddened, but did not decompose, at 300°. The glutaminic acid hydrochloride, when converted into the free acid, melted at 202°–203° C. with effervescence.

*Fraction IV.* — This fraction yielded 7.65 gm. of phenylalanine hydrochloride, 8.19 gm. of aspartic acid, 8.03 gm. of glutaminic acid hydrochloride, and 4.31 gm. of copper aspartate.

The aspartic acid, recrystallized once from water, reddened, but did not decompose, at 300°.

*Carbon and hydrogen*, 0.1450 gm. subst., gave 0.1931 gm. CO<sub>2</sub> and 0.0730 gm. H<sub>2</sub>O.

Calculated for C<sub>4</sub>H<sub>7</sub>O<sub>4</sub>N = C 36.09; H 5.26 per cent.

Found . . . . = C 36.31; H 5.59 " "

The copper salt was recrystallized from a large volume of water and air dried.

*Copper*, 0.1461 gm. subst., gave 0.0425 gm. CuO.

*Nitrogen*, 0.3535 gm. subst., required 13.2 c.c. N/10 HCl.

Calculated for C<sub>4</sub>H<sub>8</sub>O<sub>4</sub>N Cu 41/2H<sub>2</sub>O = Cu 23.07; N 5.09 per cent.

Found . . . . . = Cu 23.24; N 5.23 " "

*Fraction V.* — From this fraction there was isolated, in the same way as from the preceding fraction, 18.90 gm. of phenylalanine hydrochloride, 14.98 gm. of glutaminic acid as the barium salt, 1.70 gm. of glutaminic acid hydrochloride, and 5.78 gm. of copper aspartate.

The free glutaminic acid decomposed at about 203°.

*Carbon and hydrogen*, 0.2903 gm. subst., gave 0.4348 gm. CO<sub>2</sub> and 0.1620 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . = C 40.84; H 6.20 " "

*Fraction VI.* — By the same treatment this fraction yielded 4.41 gm. of phenylalanine hydrochloride, 27.09 gm. of glutaminic acid as the barium salt, and 2.90 gm. of glutaminic acid hydrochloride.

#### THE RESIDUE AFTER DISTILLATION.

This yielded 8.38 gm. of glutaminic acid hydrochloride, which decomposed at  $199^{\circ}$ . There were thus isolated from the total products of this hydrolysis 42.07 gm. of glutaminic acid and 22.69 gm. of glutaminic acid hydrochloride, equivalent to a total of 60.25 gm. of glutaminic acid, or 11.79 per cent of the vignin, or 69 per cent of the quantity found by Osborne and Gilbert,<sup>3</sup> who obtained 16.89 per cent by the direct method.

#### TYROSINE.

Two portions of vignin, each equal to 42.58 gm., ash and moisture free, were hydrolyzed by boiling in an oil-bath with a mixture of 150 gm. sulphuric acid and 300 c.c. of water, one for twelve and the other for twenty-three hours. After quantitatively removing the sulphuric acid with barium hydroxide, the solution was concentrated to crystallization and cooled. The products that separated when recrystallized from water weighed 0.9250 gm., and 0.9640 gm., equivalent to 2.17 and 2.26 per cent respectively.

*Carbon and hydrogen*, 0.1757 gm. subst., gave 0.3848 gm.  $\text{CO}_2$  and 0.1018 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_9\text{H}_{11}\text{O}_3\text{N}$  = C 59.67 ; H 6.08 per cent.

Found . . . . = C 59.72 ; H 6.43 " "

The filtrate from the second tyrosine estimation was used for determining the bases according to the method of Kossel and Patten,<sup>4</sup> as it was found that long continued boiling with sulphuric acid was necessary to effect complete liberation of the bases.

#### HISTIDINE.

The solution of the histidine was made up to 500 c.c.

*Nitrogen*, 50 c.c. solution required 3.56 c.c. 5/7 N—HCl = 0.3560 gm. N in 500 c.c. = 1.3136 gm. histidine = 3.08 per cent.

<sup>3</sup> OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

<sup>4</sup> KOSSEL and PATTEN: Zeitschrift für physiologische Chemie, 1903, xxxviii, p. 39.

The histidine was converted into the dichloride, which melted at  $233^{\circ}$  and gave the biuret reaction characteristic of this substance.

*Chlorine*, 0.1178 gm. subst., gave 0.1488 gm. AgCl.

Calculated for  $C_6H_9O_2N_3 \cdot 2 HCl = Cl$  31.14 per cent.

Found . . . . . = Cl 31.24 " "

#### ARGININE.

The solution of the arginine was made up to 1000 c.c.

*Nitrogen*, 50 c.c. solution required 4.73 c.c.  $5/7 N-HCl = 0.9460$  gm. N in  
1000 c.c. = 2.9392 gm. arginine + 0.1080 gm. = 3.0472 gm. = 7.20  
per cent.

The arginine was converted into the copper nitrate double salt for identification.

*Copper*, 0.2000 gm. subst., gave 0.0268 gm. CuO.

Calculated for  $(C_6H_{14}O_2N_4)_2 Cu(NO_3)_2 \cdot 3H_2O = Cu$  10.79 per cent.

Found . . . . . = Cu 10.70 " "

#### LYSINE.

The lysine picrate weighed 4.6862 gm. = 1.8243 gm. lysine = 4.28 per cent. This lysine picrate gave the following results on analysis:

*Nitrogen*, 0.3000 gm. subst., required 5.57 c.c.  $5/7 N-HCl$ .

Calculated for  $C_6H_{14}O_2N_2 \cdot C_6H_5O_7N_3 = N$  18.67 per cent.

Found . . . . . = N 18.56 " "

#### OXYPROLINE.

The residue remaining after the second esterification was freed from salts in the usual way, and then from nearly all the free hydrochloric acid, by repeatedly evaporating its solution under reduced pressure. The residue was then dissolved in about 9 litres of water, made acid with 5 per cent of sulphuric acid, and the basic amino-acids precipitated by adding a strong solution of phosphotungstic acid as long as a precipitate was formed. This precipitation was effected in three portions, about 400 gm. of phosphotungstic acid being used for each precipitation.

After removing the excess of phosphotungstic and sulphuric acid, the solution was concentrated to small volume and the chlorine removed with silver sulphate. The solution, freed from silver and sulphuric acid, when concentrated to a syrup, yielded no crystalline separation, even after long standing or continued and repeated efforts to obtain one by the addition of alcohol. The syrupy residue, after digestion with absolute alcohol and drying over sulphuric acid, became solid and practically free from fluid. In this condition it weighed 62.6 gm. It is probable, from these results, that vignin does not yield any oxyproline.

#### SERINE.

Serine was not obtained from the esters, nor from the solution, which was examined for oxyproline.

#### CYSTINE.

No attempt was made to determine cystine, on account of the small amount of sulphur contained in this protein.

The results of this hydrolysis are given in the following table:

TABLE I.

#### HYDROLYSIS OF VIGNIN.

	Per cent.		Per cent.
Glycocoll . . . . .	0.00	Oxyproline . . . . .	not found
Alanine . . . . .	0.97	Tyrosine . . . . .	2.26
Valine . . . . .	0.34	Cystine . . . . .	not determined
Leucine . . . . .	7.82	Arginine . . . . .	7.20
Proline . . . . .	5.25	Histidine . . . . .	3.08
Phenylalanine . . . . .	5.27	Lysine . . . . .	4.28
Aspartic acid . . . . .	3.97	Ammonia . . . . .	2.32
Glutaminic acid . . . . .	16.89	Tryptophane . . . . .	present
Serine . . . . .	not found	Total . . . . .	59.65

The results of this hydrolysis are similar to those found for the proteins of other leguminous seeds, *e. g.*, legumin, vicilin, phaseolin and glycinin. Although these proteins are in many respects much alike, sufficiently positive differences, of one kind or another, exist between them to leave no doubt that each is a distinctly different protein.

In conducting this hydrolysis, care was taken to keep account of the undetermined substance, in order to get a clearer idea of its approximate amount, and if possible to locate the large loss indicated by the low summation.

The substances making up the above total are stated as the free amino-acids, and, in addition, a small amount of ammonia. The amino-acids are doubtless united in the protein molecule with the elimination of a molecule of water for each molecule of acid, and it is not improbable that the ammonia is combined with one carboxyl group of the dibasic acids.<sup>5</sup>

Assuming these combinations, we have calculated the amount of each of the substances determined, as it is supposed to occur in the protein molecule. The results are as follows:

TABLE II.

PROPORTION OF THE AMINO-ACIDS AS THEY ARE ASSUMED TO BE COMBINED IN THE MOLECULE OF VIGNIN.

	Per cent.		Per cent.
Glycocoll . . . . .	0.00	Glutaminic acid . . . . .	12.84
Alanine . . . . .	0.78	Tyrosine . . . . .	2.03
Valine . . . . .	0.29	Arginine . . . . .	6.04
Leucine . . . . .	6.73	Histidine . . . . .	2.71
Proline . . . . .	4.41	Lysine . . . . .	3.77
Phenylalanine . . . . .	4.69	Ammonia . . . . .	2.18
Aspartic acid . . . . .	2.94	Total . . . . .	49.41

In separating the amino-acids from each other, a certain quantity always results which is a mixture of two or more acids that cannot be separated into products sufficiently pure to weigh. In this hydrolysis, the total weight of such mixtures, obtained from the esters distilling over below 100°, was 21.9 gm., or 4.10 per cent of the vignin. This loss falls chiefly on alanine, valine, leucine, and possibly isoleucine.

From the fractions of the esters distilling above 100° a considerable quantity of substance is always obtained, which remains as a syrup, after separating phenylalanine, aspartic acid, glutaminic acid, and serine, the nature of which cannot be determined by any method at present known. As this syrup cannot be reduced to a condition suitable for weighing, we have calculated its approximate amount by assuming that the esters would yield on saponification the same

<sup>5</sup> Cf. OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

amount of amino-acids as does glutaminic acid ester, *i. e.*, 72 per cent of the weight of the ester.

By this method we find that the esters, distilling above 100°, would yield 122.83 gm., from which there was separated, in weighable condition, 102.35 gm. of substance, making the quantity of undetermined matter 20.48 gm., or 4.01 per cent of the vignin.

The total loss in separating the amino-acids obtained from the esters was 8.11 per cent of the vignin. Assuming this to consist of amino-acids combined with one another and having a mean molecular weight equal to that of leucine, this quantity is equal to 6.98 per cent of the vignin.

From the undistilled residue of the esters, which weighed 45 gm., there was obtained 6.71 gm. of glutaminic acid, much, if not all, of which was present as the ester. Deducting the corresponding weight of ester, 9.27 gm., from that of the residue, 45 gm., we have 35.73 gm. of undetermined substance in the undistilled residue, or 7 per cent of the vignin.

This 7 per cent of undetermined matter consists, to some extent, of diketopiperazines formed by condensation of the amino esters with elimination of alcohol, but nothing definite can be said concerning the nature of other substances present in it.

Summing up the calculations, we have:

	Per cent
Amino-acids which were weighed as definite substances, calculated as combined in the protein . . . . .	49.41
Amino-acids which were weighed as undefined mixtures, calculated as combined in the protein . . . . .	6.98
Undistilled residue . . . . .	7.00
Total . . . . .	63.39

We thus have, approximately, 63 per cent, which for the most part probably consists of amino-acids which are at present known to be decomposition products of the proteins.

A similar but somewhat higher result is given by a different method of calculation, as shown by the following:

The sum of the amino-acids as given in Table II, obtained by distilling the esters, is 19.84 per cent of the vignin. Assuming that all of the amino-acids which were obtained from the esters as mixtures that could not be weighed as definite substances were com-



posed of the same amino-acids as those weighed, and that one half of the undistilled residue consisted of anhydrides of these same acids or the free acids themselves, we have 30.31 per cent of the protein in the form of such acids as are commonly estimated by distilling the esters. If it is assumed that in esterifying 80 per cent of the amino-acids are obtained as esters, this 30.31 per cent would be equal to 37.89 per cent of the protein and this latter quantity would then represent the proportion of these amino-acids originally yielded by hydrolysis. Assuming further that the results of the direct determinations of the other products of hydrolysis are correct, and adding their sum to the preceding figure, we have 67.46 per cent of the protein as possibly composed of the substances enumerated in the analysis. The following table gives the details of the above calculation:

TABLE IV.

	Per cent.	Per cent.	Per cent.
Amino-acids obtained from the esters and weighed.	Alanine . . . . .	0.78	
	Valine . . . . .	0.29	
	Leucine . . . . .	6.73	
	Proline . . . . .	4.41	
	Phenylalanine . . . . .	4.69	
	Aspartic acid . . . . .	2.94	
	Amino-acid mixture not separated but weighed . . . . .	6.98	
	One half of undistilled residue . . . . .	3.49	
	Twenty per cent of original acids, added on account of incomplete esterification . . . . .		7.58
Directly determined.	Glutaminic acid . . . . .	12.84	
	Tyrosine . . . . .	2.03	
	Arginine . . . . .	6.04	
	Histidine . . . . .	2.71	
	Lysine . . . . .	3.77	
	Ammonia . . . . .	2.18	
	Total . . . . .		67.46

We thus have, at the least, 30 per cent of the vignin which is not accounted for in the above table. The substance obtained from the unesterified and ether insoluble part of the products of hydrolysis, which was examined for oxyproline, weighed only 62.6 gm., equal to 12.26 per cent of the vignin. Of this, 7.58 per cent is included in the 20 per cent of unesterified amino-acids, leaving only 4.68 per

cent for unknown substances. It would seem from these figures that the losses which are practically unavoidable in carrying out these analyses may have a larger share in explaining the deficiency than has been heretofore supposed.

It does not, however, seem probable that this apparently large deficiency consists to any considerable extent of products of decomposition already known. The amount of the substances that are determined directly, probably nearly represents the quantity in which they are produced by hydrolysis, for the determinations of glutaminic acid can be controlled to a certain extent by the results obtained by the ester method, and those of arginine, histidine, and lysine are, as will later be shown, accurately controlled by the nitrogen precipitated by phosphotungstic acid. The ammonia determinations are accurate.

The known protein decomposition products which were not determined in this analysis cannot be relied on to account for this difference, for the presence of much diamino-trioxydodecanic acid would, presumably, be shown by the excess of nitrogen precipitated by phosphotungstic acid over that contained in arginine, histidine, and lysine; oxyproline and serine could not be found, and were certainly not present in large amounts; isoleucine was weighed in the mixture of unseparated acids; and the amount of cystine could not exceed 2 per cent if all the sulphur of this protein were present in this substance. Tryptophane, therefore, remains as the only known substance which could make up any considerable amount of this undetermined part of the protein. It is, however, not at all probable that any large quantity of tryptophane is present in this and other proteins yielding similar analyses. It might be thought that carbohydrates are among the still unrecognized products of these protein hydrolyses, but this is rendered improbable by the fact that the analyses of those proteins which give no Molisch reaction show the same relatively low summation as those giving this reaction, and that there are better reasons for considering the Molisch reaction, that is given by most proteins, to be due to contamination of the preparations, than to a carbohydrate constituent of their molecules. We may, therefore, expect to find among the decomposition products of proteins still unrecognized substances.

## STUDIES IN EXPERIMENTAL GLYCOSURIA.—II. SOME EXPERIMENTS BEARING ON THE NATURE OF THE GLYCOGENOLYTIC FIBRES IN THE GREAT SPLANCHNIC NERVE.

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**I**N a previous communication,<sup>1</sup> it was shown that when every precaution is taken against asphyxia hyperglycæmia does not result from stimulation of the central end of the vagus nerve or of the uncut spinal cord, in well-fed dogs anæsthetized with pure ether. In other words, it was found impossible to demonstrate, in these positions, the existence of afferent or efferent nerve fibres connecting with the hypothetical diabetic centre in the medulla oblongata.

It was further shown that stimulation of the greater splanchnic nerve on the left side in such animals is almost invariably followed by hyperglycæmia, although this result was not obtained when the greater splanchnic nerves on both sides had been cut and the blood pressure therefore considerably lowered.

In the present communication, more complete data will be offered concerning the amount of blood sugar, the rate of diuresis, and the extent of glycosuria following splanchnic stimulation; after which several experiments will be recorded bearing on the question of the exact nature of the fibres the stimulation of which in the splanchnic nerve brings about these results.

The influence of faradic stimulation of the left greater splanchnic nerve on the amount of sugar in the blood, the rate of urine excretion and the amount of sugar in the urine.

The technique of the experiments here recorded was as follows. Dogs, usually fed the day before the experiment with bread and meat, were anæsthetized with pure ether, a tracheal cannula inserted

<sup>1</sup> Cf. MACLEOD, J. J. R.: This journal, 1907, xix, p. 388.

and in most cases a stream of washed oxygen allowed to perflate one lung through a rubber catheter passed down the respiration tube as far into the bronchi as possible. Cannulæ were then introduced into the carotid and femoral arteries; the former was connected with a mercury manometer, magnesium sulphate solution being used as anticoagulant; the latter was employed for removing samples of blood for analysis. Cannulæ were also introduced in the ureters for the collection of urine. The greater splanchnic nerve was then exposed on the left side and electrodes placed in position on it, after which the abdominal wound was closed, and a weighed sample of blood removed through the femoral cannula for estimation of the sugar by the method of Waymouth Reid.<sup>2</sup>

In the above procedure, objection may be taken to the use of magnesium sulphate solution as an anticoagulant on the ground that the introduction of any of it into the artery, as must occur with the changes in blood pressure caused by stimulation of the splanchnic nerve, will act on the respiratory centre and weaken the natural respiratory movements, thus tending to cause hyperglycæmia as a result of an asphyxial condition.<sup>2</sup> Many experiments in which no hyperglycæmia became established despite considerable changes in blood pressure show, however, that with an abundant supply of oxygen any error on this score can be discounted. (See Table V.) In this connection it might be well to state that my reason for recording the general arterial blood pressure was that it furnishes the best evidence of successful application of the electrodes (*e. g.*, to the splanchnic), and of the conditions of the circulation on which depends to a large extent the results of the experiment.

In these experiments, as in the previous ones, I have chosen 0.2 per cent of sugar in the blood as that above which a condition of hyperglycæmia is to be considered as present. This is undoubtedly itself an abnormally high percentage, but inasmuch as the normal percentage of sugar in the blood (as determined by Waymouth Reid's method) of dogs merely kept under ether for several hours is somewhat variable, and has not been made the object of special study, it was thought better to err on the safe side in these experiments by choosing a high normal value. According to Liefmann

<sup>2</sup> MELTZER, S. J., and AUER JOHN: This journal, 1906, xv, p. 387. It is important to note, however, that the *gradual* injection of magnesium salts does not, according to these authors, have any effect on respiration. UNDERHILL, F. P., and CLOSSON, O. E.: This journal, 1906, xv, p. 330.

and Stern<sup>3</sup> the sugar content of the blood of man under normal conditions varies from 0.065 to 0.105 when Schenck's method of analysis is employed. By this latter method, however, it is probable that the results are somewhat lower than by Reid's method. Embden, Lüthje, and Liefmann<sup>4</sup> by the same method determined the amount of sugar in the blood removed from the external jugular vein of dogs kept in rooms at different temperatures. They found the percentage amount to vary from 0.064 in the case of a dog kept at 30° C. to 0.106 in one kept at 10° C. The amount of blood sugar varied inversely as the temperature of the environment.

The experiments of which the results are given in Table I were performed with the object of furnishing further evidence regarding the relative frequency of positive and negative results. Of the six, only one (*viz.*, No. 19) gave a negative result, *i. e.*, no hyperglycæmia. Although the blood samples were not as a rule removed until about one hour after starting the stimulation of the nerve, yet, in the cases of Experiments 1, 2, and 3 at least, hyperglycæmia must have been present before this time, as evidenced by the appearance of sugar in the urine and by diuresis. In all of these experiments oxygen was freely administered, and the animals were liberally fed with bread and meat for at least a day previous to that of the experiment.

The percentage of reducing substance in the urine and the number of cubic centimetres of urine excreted per minute will be seen to have increased to a corresponding degree in the case of the first three of these experiments. As this parallelism was more striking in the experiments about to be described we will defer its consideration for the present.

In the experiments of the second table the same general procedure was followed as in those of the first; although oxygen was given in only one case (No. 5). In two of them (Nos. 5 and 6) the stimulation of the splanchnic nerve was stopped after hyperglycæmia had become well established; in another case (No. 7) it was kept up for nearly five hours; and in the remaining one of the series (No. 8) the greater splanchnic nerve was cut on the left side before the electrodes were applied.

Regarding for the present the changes in the percentage of re-

<sup>3</sup> LIEFMANN and STERN: *Biochemisches Zeitschrift*, 1906, i, p. 299.

<sup>4</sup> EMBDEN, G., LÜTHJE, and LIEFMANN: *Beiträge zur chemischen Physiologie und Pathologie*, 1907, x, p. 265.

TABLE I.  
STIMULATION OF LEFT GREAT SPALANCHNIC NERVE IN DOGS RESPIRING OXYGEN-RICH AIR.

No. of expt.	Wt. of dog.	Per cent of reducing substance in blood.		Per cent of reducing substance in urine.	Urine excreted per minute in cubic centimeters. <sup>1</sup>	Remarks.
		Before.	- After.			
1	10.075	0.231 (Electrodes had been <i>in situ</i> for 35 min.)	83 min. — 0.231	33 min. — doubtful. 60 min. — 1.5 80 min. — 2.5	0.10 0.14 0.36	Oxygen; fed for two days with bread and meat; b. p. at start 90–100 mm.; no great rise on stimulating.
2	....	....	....	15 min. — trace 35 min. — 2.2 50 min. — 2.64	0.62 1.07 1.26	Oxygen; fed for two days with bread and meat; b. p. 105 rising to 160 on stimulating splanchnics; afterward the rise was less marked.
3	17.20	....	....	8 min. — no urine collected. 28 min. — 0.326 58 min. — 1.17 75 min. — 3.57	.. 0.26 0.22 0.56	Oxygen; fed for one day with bread and meat; b. p. 95–100 mm. distinctly rising with stimulation. Enlarged thyroid.
4	15.600	....	72 min. — 0.271 60 min. — 0.219	40 min. — sugar free. 60 min. — sugar in urine.	0.26	Oxygen; for some time during this experiment a blood vessel was being stimulated instead of the nerve; thyroid enlarged; fed as above.
19	12.90	0.160	60 min. — 0.174 120 min. — 0.114	Doubtful trace.	Only a few drops during entire experiment.	Oxygen and artificial respiration from start; not specially fed; b. p. 110 mm.; moderate rise on stimulation.
20	12.00	0.151	130 min. — 0.216 150 min. — 0.255	Considerable amount not estimated.	No marked diuresis.	Oxygen and artificial respiration from start; fed as above.

<sup>1</sup> The time periods of collection of samples are given in corresponding line of 5th column.

ducing substance in the blood, it will be seen that, when stimulation was kept up for a long period of time, this attained its maximum in about two hours, after which it fell off slowly (No. 7), being still high after five hours. Removal of the stimulation did not appear to accelerate the diminution of reducing power, for in one experiment (No. 5) this was still 0.301 per cent in 187 minutes after removal of the stimulus, the maximum during stimulation having been 0.348; and in another case (No. 6), it was 0.180 in 185 minutes after removal of the stimulus, the maximum having been 0.232 per cent. In the one case the percentage fell from 0.348 to 0.301 (0.048 per cent) in 187 minutes; and in the other, from 0.232 to 0.180 (0.052 per cent) in 185 minutes. A comparison of the rate of diminution of the blood sugar in these experiments reveals a result which is undoubtedly accidental, *viz.*, that it is greater in the cases where the stimulation was maintained throughout, than in those where the stimulus was removed.

From so small a number of observations it would, of course, be dangerous to draw any final conclusions, but the experiments at least suggest two probabilities; first, that the effect of stimulation of the great splanchnic nerve on the amount of sugar in the blood is maintained for some considerable time after the stimulus itself has been removed, and secondly, that the mechanism involved begins to show exhaustion in about two hours. The cause of the lessened effect of stimulation of the splanchnic nerve on the percentage of reducing substance in the blood lies no doubt mainly in the gradual disappearance of its source of supply in the liver, *i. e.*, the glycogen. Some experiments bearing on this part of the subject will be reported in a succeeding communication. Another cause must, however, be considered as possibly contributing to the decline, *viz.*, fatigue of the stimulated nerve. That such fatigue does appear after prolonged stimulation of the splanchnic nerve was evidenced by the less marked effect of the stimulation on the blood pressure after the experiment had been in progress for about one to one and a half hours. Sometimes this lessening of effect was quite marked, and it was always more or less present. Since nerve fibres both medullated and non-medullated are indefatigable,<sup>5</sup> the seat of fatigue in the above case must be either in the coeliac ganglia or locally at the seat of application of the electrodes ("stimulation fatigue").

<sup>5</sup> HALLIBURTON: *Biochemistry of muscle and nerve*, Philadelphia, 1904, p. 89; HOWELL, W. H., BUDGETT, L. P., and LEONARD, E.: *The journal of physiology*, 1894, xvi, p. 298.

TABLE II.  
STIMULATION OF SPALANCHNIC NERVE FOR SEVERAL HOURS OR ONLY DURING FIRST PART OF EXPERIMENT.

No. of expt.	Wt. of dog.	Per cent of reducing substance in blood.		Per cent of reducing substance in urine.	Urine excreted per minute in cubic centimeters. <sup>1</sup>	Remarks.
		Before.	After.			
7	9.200	0.141	55 min. —	20 min. — none.	0.07	No oxygen; fed with cooked meat and soup for two days before; b. p. 140 mm.; well marked b. p. reaction on stimulation; stimulation kept up off and on all the time; rectal temperature at end 32° C.
			115 min. —	50 min. — 0.87	0.1	
			235 min. —	80 min. — 2.85	0.12	
			295 min. —	110 min. — 3.3	0.17	
			105 min. —	170 min. — 3.3	(0.08)	
5	7.500	.....	235 min. —	230 min. — 1.00	0.14	Oxygen; fed for two days before on bread and meat; b. p. 110 mm.; reacted well to stimulation; b. p. fell on account of hemorrhage.
			295 min. —	290 min. — 0.54	0.09	
			25 min. —	15 min. — 0.5	0.21	
			105 min. —	46 min. — 1.26	0.52	
			.....	76 min. — 2.33	.....	
6	9.400	.....	.....	106 min. — 1.61	1.04	No oxygen; b. p. 140 mm.; rose to 170 on stimulation; protein in urine; not specially fed.
			.....	(stood two days).	.....	
			.....	Stimulus off.	.....	
			.....	After	0.015	
			.....	132 min. — 2.25	0.06	
8	.....	.....	187 min. —	192 min. — 2.25	0.48	No oxygen; fed as above; left splanchnic cut; b. p. at start 100 mm.; marked reaction on stimulation of splanchnic; breathing unaffected; MgSO <sub>4</sub> sol. entered circulation.
			125 min. —	197 min. — 2.17	.....	
			.....	35 min. — none.	0.12	
			.....	65 min. — trace.	0.10	
			.....	95 min. — 1.4	0.13	
			125 min. —	125 min. — 1.66	.....	
			.....	Stimulus off.	.....	
			.....	After	0.06	
			.....	60 min. — 1.66	0.05	
			.....	120 min. — trace.	0.05	
			185 min. —	185 min. — trace.	.....	
			.....	Bladder urine sugar free.	.....	
			.....	20 min. — 0.45	0.47	
			.....	40 min. — 1.4	0.22	
			.....	60 min. — 1.6	0.22	
			90 min. —	90 min. — 2.2	0.16	
			.....	.....	.....	
			.....	.....	.....	
			.....	.....	.....	
			.....	.....	.....	

<sup>1</sup> The time periods of collection of samples are given on corresponding line of 5th column.



From the experiments recorded in Table II, it will be seen that the rate of urine excretion (in cubic centimetres per minute) and the percentage of reducing substance in the urine ran approximately parallel with the percentage of reducing substance in the blood. This fact is most clearly shown in Experiment 7, in which a sufficient number of data are furnished from which it is possible to plot a curve as has been done in Fig. 1. There can be no doubt from such results that the cause of the diuresis and glycosuria is the hyperglycæmia.

In certain experiments dealing with the effect of asphyxia itself on these three values I have found a similar relationship between them. Thus in No. 7 a dog weighing 9.2 kg. gave, for percentage of blood sugar (after 115 minutes' stimulation) 0.400, the rate of urine excretion per minute being 0.17 c.c., and the percentage of dextrose 3.3.; in an experiment in which a dog of 8.7 kg. was partially asphyxiated by clamping the tracheal cannula the percentage of blood sugar was 0.374 after 90 minutes, the urine excretion 0.35 c.c. per minute, and the percentage of dextrose in this 2. The diuretic influence of dextrose is of course well known, but in the above experiments it was at first thought that possibly another factor might be held accountable for the large excretion of urine, *viz.*, the periodic changes in the volume of the kidney. During the periods of stimulation, which lasted for about one minute, the kidney volume diminishes, to enlarge again during the periods of rest, which lasted for about two minutes; and it was thought that these changes in volume might, so to say, confer a pumping mechanism on the organ and thus accelerate urine excretion. It should be pointed out in this connection that, although intense asphyxial hyperglycæmia may cause a diuresis and glycosuria similar to that observed above, yet I have never observed such to follow stimulation of sensory nerves (*e. g.*, vagus) acting on the respiratory centre. This fact is of interest in showing us that the hyperglycæmia following stimulation of the splanchnic nerve cannot be due to afferent stimulation of the respiratory centre, as for example might occur through spread of stimulus to the vagus terminations in the celiac plexus.

When nervous control of the production of sugar by the liver was discovered by Claude Bernard's well-known piqure experiments, the existence of nerve fibres having a specific influence over the secreting functions of gland cells was unknown. Heidenhain's experiment on the existence of saliva-secretory fibres in the chorda tym-

pani had not yet been performed, and it was believed that the activity of glandular secretion depended solely on conditions of local blood supply. It was therefore taught by Bernard, and his teaching in this regard has been generally accepted, that the hyperglycogenolysis following piqûre is due to an increased blood supply through the liver. To quote Bernard:<sup>6</sup> "Si l'on examine l'état des viscères abdominaux chez un animal qui a subi la piqûre diabétique, on voit que la circulation y est considérablement activée.

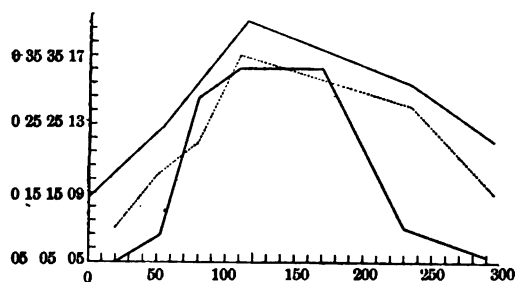


FIGURE 1. — Curves compiled from results of Exp. 7 (Table II) showing relationship of per cent of reducing substance in blood (thin continuous line) to that of urine (thick continuous line) and amount of urine formed per minute (broken line).

. . . L'augmentation de rapidité de la circulation du foie accroit la glycémie. . . . Les cellules hépatiques foyers de matière glycogène, se trouvent entourées, d'une sorte de réseau sanguin; la circulation devenant plus active dans le réseau, le contact du liquide sanguin avec les liquides cellulaires mieux assuré, l'action est plus énergique sur la matière glycogène, la transformation devient plus abondante, et le sucre produit est immédiatement entraîne."

This view of the mechanism has been accepted, not only for piqûre, but also for all those cases in which, by nerve stimulation, a similar hyperglycæmia is induced. The question therefore arises as to *whether the hyperglycogenolysis following splanchnic nerve stimulation can likewise be accounted for by vascular changes, or whether this nerve may not contain specific secretory fibres controlling the production or the activity of hepatic glycogenase.*

When the vascular disturbance leads, as Claude Bernard claims it does in the case of piqûre, to a more rapid circulation through the hepatic lobule, then a flushing out of sugar from this may be assumed; but if the vascular change be in the opposite direction, *viz.*, a diminution of blood supply, as is the case when the splanchnic nerve is stimulated, then we must consider the hepatic cells as re-

<sup>6</sup> CLAUDE BERNARD: *Leçons sur le diabète*, Paris, Bellaire et Fils, 1877, p. 371.

acting to diminished blood supply by increasing their glycogenolytic function. The invariable increase of sugar in the blood as a result of asphyxia, however produced, would suggest the most probable explanation for the increased glycogenolysis as being a direct action of the blood on the liver cell. The two salient properties of asphyxial, as contrasted with normal blood, are a deficiency of oxygen and an excess of carbonic acid, either one of which, or both, may therefore be considered as specific stimulants of the glycogenolytic activities of the liver cell.

Diminished blood supply to the hepatic lobule must result from stimulation of the great splanchnic nerve, and indeed in two ways: first, by less blood getting through the constricted splanchnic vessels, and secondly, because of the constriction in the intrahepatic portions of the portal vein and hepatic artery. This diminished vascularity of the liver may lead to the same reaction on the part of its cells as is invoked by asphyxial blood; in other words, we may have a *local asphyxia of the hepatic cells*. It is, however, difficult to see how the activity of the liver lobule can depend very much on the arterial condition of the blood, for the arterial blood carried into it by the hepatic arteries is relatively very much smaller than the already venous blood carried to it by the portal vein. The glycogenolytic activity of the hepatic cells must be considered as being peculiarly reactive towards changes in blood composition; when the blood contains the normal amount of oxygen and carbonic acid, it is only the percentage of dextrose in it which influences sugar production by the liver, but when abnormal amounts of these gases are present, as in asphyxia, the controlling influence of dextrose content evidently falls into abeyance and a new stimulus appears.

In the foregoing argument it is assumed that the action of asphyxial blood in producing hyperglycæmia is a direct one on the hepatic cell. From all the experiments which have been performed on asphyxial glycosuria it might just as well be, however, that the seat of action of the asphyxial blood is not the hepatic cell itself but the nerve centres. When the great splanchnic nerve is stimulated, therefore, the hyperglycæmia which results might be due to asphyxia of the hepatic cells, or of the nerve cells of the celiac plexus.

In a recent series of papers, Ivar Bang, Ljungdahl, and Böhm<sup>7</sup>

<sup>7</sup> BANG, LJUNGDAHL, and BÖHM: Beiträge zur chemischen Physiologie und Pathologie, 1907, ix, p. 408; 1907, x, p. 1; 1907, x, p. 312.

have recorded numerous observations on the amount of glycogenolytic ferment (glycogenase) contained in the liver of rabbits after various experimental procedures calculated to influence its amount. The technique of these experiments was as follows: after bringing about some experimental condition, such as piqûre, stimulation of the central end of the vagi, saline infusion, etc., the animal was killed with ether and the liver immediately excised and rapidly transfused through the portal vein with 0.8 per cent sodium chloride solution. When the washings had become colorless, the liver was minced up and divided into two portions, each of which was mixed with an equal volume of 0.8 per cent NaCl solution. One of these was heated to boiling, so as to destroy its ferment. It was then placed along with the other unheated portion in an incubator at 37° C. for four hours, at the end of which period the percentage amount of glycogen was determined in the two portions by Bang's modification of Pflüger's process. From the difference in the amount of glycogen found in these two portions, the percentage amount which had undergone hydrolysis was calculated, and this was taken as an index of the amount of glycogenolytic ferment.

It was found that, under similar conditions, the amount of ferment, as determined in this manner, was fairly constant. The percentile change in four hours for the liver of animals killed by ether narcosis amounted on an average to 6.3; in cases where the liver had been perfused with warm solutions of sodium chloride previous to death this value rose to about 13 when isotonic solutions were used, or much higher with hypisotonic or cooled solutions. Asphyxia of the animal previous to removal of the liver caused the value to rise to 18. The hypersecretion of the ferment as a result of saline transfusion can therefore probably be ascribed to asphyxia. When the liver was removed immediately after piqûre, a great increase was found in the ferment, but if some time had elapsed after the piqûre before removal of the liver, there was a deficiency of ferment. Cutting of the vagi led to an increased amount of ferment after some time.

These experiments are of intense interest to us in connection with our present discussion. They show us that a flushing out of sugar from the liver as a result of vasodilatation cannot be the explanation of the hyperglycogenolysis even in the case of piqûre, and further they show us that the amount of glycogenolytic ferment is increased whenever an asphyxial condition becomes established in

the liver. Whether the observations on piqure warrant the conclusions drawn by the authors from their results that the nervous system *per se* exercises a control on the ferment production by the liver, we will leave for the present undecided.

Returning now to our present question, *viz.*, the nature of the glycogenolytic fibres in the splanchnic nerve, there can be found in the literature only one reference to any experiments dealing with it. These are by the Cavazzani brothers.<sup>8</sup> By estimation of the sugar content of blood removed from the hepatic veins they found that stimulation of the coeliac plexus is followed by increased output of sugar by the liver. To ascertain whether this is due to stimulation of secretory fibres proper, they removed a piece of liver from dogs immediately after death and extracted its sugar by boiling water; the coeliac plexus was then stimulated for fifteen minutes, after which the sugar content of the remaining portion of liver was similarly estimated. They found a great increase in the latter case and concluded that true secretory fibres must be present, since vascular changes were precluded by there being no circulation of blood present. Apart from the technique of estimation of sugar, of which no details are given in any of the papers, but which, judging from the variable results recorded in the first quoted paper, seems to have been faulty, the research really gives us no evidence either one way or the other; for no comparative figures are given of the increase of sugar in cases where the liver is merely left *in situ* in the body without any nerve stimulation.

From a consideration of the above literature, the question in the present research narrows itself down to this: Is the hyperglycogenolysis following stimulation of the greater splanchnic nerve due to the presence in these nerves of true glycogenase secretory fibres, or is it a result of the diminished blood supply to the liver or coeliac ganglion nerve cells induced by stimulation of vasoconstrictor fibres?<sup>9</sup>

So far the following experiments have been performed in solution of this problem:

<sup>8</sup> CAVAZZANI (frères): Archives italiennes de biologie, 1893, xix, p. 270; CAVAZZANI: Centralblatt für Physiologie, 1894, viii, p. 32; CAVAZZANI, EMIL: Archives für die gesammte Physiologie, 1894, lvii, p. 181.

<sup>9</sup> The possibility of afferent stimulation of the respiratory centre has already been considered above and found untenable. Further justification for this conclusion is obtained in the results of the experiments reported in Tables V and VI.

Estimation of the amount of reducing substance in the blood before and after:—

(a) Interference with blood supply to the liver without stimulation of nerves.

(b) Stimulation of splanchnic nerves after cutting the hepatic nerves.

(c) Stimulation of the hepatic nerves near the hilus of the liver.

(d) Stimulation of the splanchnic nerves after the administration of atropin.

The value of each of these experiments in connection with the present question will be considered when discussing their respective results.

The amount of reducing substance in the blood as influenced by interference with the blood supply to the liver.

The portal vein and the hepatic artery carry blood to the liver lobule for very different purposes; the former in order to have certain blood constituents acted upon by the hepatic cells, and the latter to maintain the nutrition of the organ. The oxygen supply must be derived from the blood of the hepatic artery, for there can be little of this available in the portal blood. Very little, if any, of this oxygen is required for the various processes of synthesis and analysis performed by the hepatic cells, but it is necessary for the life of these cells.

It is commonly believed that it is deprivation of oxygen rather than excess of carbonic acid which is the immediate cause of asphyxial glycosuria. The fact that inhalation of pure oxygen prevents the appearance of glycosuria when pulmonary ventilation is interfered with, as well as after the administration of certain drugs which otherwise tend to produce asphyxia, is one of the most important supports for this belief.<sup>10</sup>

It is not known whether the hyperglycæmia in asphyxial conditions is due entirely to increased hepatic glycogenolysis, or whether it may not also be due, partly at least, to diminished glycolysis. Thus it is suggested by Underhill that deficiency of oxygen depresses the activity of oxidases, and thus tends to cause accumulation of dextrose in the blood.<sup>11</sup> If diminution of oxygen supply to the liver should stimulate the breakdown of glycogen, then the constriction of the hepatic arteries which must of necessity follow stimulation

<sup>10</sup> MACLEOD, J. J. R.: Communication 1, *loc. cit.*

<sup>11</sup> UNDERHILL, F. P.: The journal of biological chemistry, 1905, i, p. 113.

TABLE III.  
LIGATION OF HEPATIC ARTERY.<sup>1</sup>

No. of expt.	Wt. of dog.	Per cent of reducing substance in blood.		Per cent of reducing substance in urine.	Urine excreted per minute in cubic centimetres. <sup>2</sup>	Remarks.
		Before.	After.			
21	....	0.182	60 min. — 0.186 80 min. — 0.211	..... Considerable amount.	..... Excretion small.	Oxygen; bladder urine contained some reducing substance.
22	....	....	5 min. — 0.124 125 min. — 0.132 135 min. — { 0.139 0.121	30 min. — no sugar. 60 min. — no sugar. 70 min. — trace.	0.08 0.03 For remainder small amount.	Oxygen; fed with flesh and bread on day before. <i>Post mortem</i> ligature correctly placed.
23	....	....	5 min. — 0.152 — { 0.144 — { 0.149 85 min. — { 0.140	No ureter cannulas.	.....	Oxygen; <i>post mortem</i> ligature correctly placed.
24	12.70	....	5 min. — 0.157 65 min. — 0.167 105 min. — 0.165	30 min. — trace. 60 min. — trace.	..... 0.08 0.07	Oxygen; <i>post mortem</i> ligature correctly placed.

<sup>1</sup> The ligature was applied to the main hepatic artery central to where the hepatic branches are given off.

<sup>2</sup> The time periods of collection of samples are given in the corresponding lines of preceding column.

of the splanchnic nerves might be the explanation of the hyperglycæmia. It was deemed important, therefore, to see whether ligation of the hepatic arteries causes hyperglycæmia. Several experiments of this nature were performed on well-fed dogs, the hepatic artery being ligated before the hepatic branches proper are given off. Ligation in this position of course cuts off the blood supply to part of the pancreas and duodenum, but this can in no way interfere with the value of the results obtained.

It will be seen that in no case did the ligation lead to hyperglycæmia. We may conclude, therefore, that constriction of the hepatic artery is not the cause of the hyperglycogenolysis which follows stimulation of the greater splanchnic nerve.

Should vascular disturbance be the cause of the hyperglycogenolysis its seat of action must therefore be on the portal vein. That vaso-motor fibres to the hepatic end of the portal vein are contained in the great splanchnic nerves has been shown by Mall,<sup>12</sup> who found that after ligation of the thoracic aorta and portal vein before its entry to the liver, stimulation of the splanchnic nerves still caused a rise in carotid blood pressure. Mall also isolated the hepatic nerves and found, when the nerves were not cut, that a slight rise in carotid blood pressure was produced on stimulating them. When the nerves were cut and the aorta clamped, stimulation produced a slight rise in three experiments, and no effect, or a slight fall, in one experiment. With open aorta this last-mentioned experiment gave a slight rise after about thirty seconds. These nerve fibres have also been demonstrated by Bayliss and Starling,<sup>13</sup> who, by determining the pressure in the portal vein (*i. e.*, central end of splenic vein), found on stimulating the third to the eleventh spinal roots that a rise occurred in this, even after the preliminary rise and fall in pressure caused by constriction of the mesenteric vessels had subsided. This preliminary effect due to mesenteric constriction became more marked with the lower roots. Cavazzani and Manca<sup>14</sup> have confirmed these results by perfusing warm physiological saline through the liver and measuring the outflow before and during splanchnic stimulation; so have François-Franck and Hallion<sup>15</sup> by the use of the plethymographic method.

<sup>12</sup> MALL: *Archiv für Physiologie*, 1892, p. 409.

<sup>13</sup> BAYLISS and STARLING: *The journal of physiology*, 1894-1895, xvii, p. 120.

<sup>14</sup> CAVAZZANI and MANCA: *Archives italiennes de biologie*, 1895, xxiv, p. 33.

<sup>15</sup> FRANÇOIS-FRANCK and HALLION: *Archives de physiologie*, 1897, pp. 434-448.



Now, although a curtailment of portal blood supply by vasoconstriction can scarcely be considered as leading to an asphyxial condition in the ordinary sense of the word, yet it may be that a change in this supply acts as a stimulus to glycogenolysis; that is to say, that changes in the pressure or the volume of the blood flow may *per se* excite the glycogenolytic process.

I have attempted to throw some light on the influence of changes in portal blood supply on sugar production by clamping the portal vein for short periods of time just before its entry to the liver, or, in one case (Table IV, No. 25), by continuously constricting the vein. In another case the hepatic arteries were also ligated (No. 46).

Examination of Table IV, which gives the results of these experiments, will show that no increase in blood sugar was caused in one experiment (No. 26), only moderate increase in two others (Nos. 25 and 27), and marked increase in the remaining two (Nos. 46 and 48). In the last two cases, the dogs were fed with considerable quantities of cane sugar some time (16 hours) prior to the experiment, and in the other cases with large quantities of flesh. The large values for the percentage of sugar in normal blood seen in this table, as also in Table III and V, are probably to be accounted for by the handling of the liver which was involved in the necessary operative manipulations. In No. 27, the urine collected during the preliminary operations contained a large amount of sugar.

There can be no doubt from these results that considerable interference with the portal blood supply causes hyperglycogenolysis, which, however, does not occur to any marked extent when the interference is only moderate in degree. When the portal blood supply is cut off for more than about two minutes it seems that a process analogous with post mortem glycogenolysis sets in.

Diversion of the portal blood into the vena cava by the establishment of Eck's fistula does not cause reducing substance to appear in the urine. The animals do not pass much urine for several days after the operation, but what is passed has not been noted by any of the observers of this condition to contain sugar.<sup>16</sup>

This fact may at first sight seem to stand in contradiction with the results obtained by clamping the portal vein, but, if we consider

<sup>16</sup> MACLEOD, J. J. R.: Aberdeen University Quatercentenary Publications, 1906, p. 267; HAWK, P.: private communication; DE FILLIPPI, F.: *Zeitschrift für Biologie*, 1907, xlix, p. 511.

TABLE IV.  
CLAMPING PORTAL VEIN.

No. of expt.	Wt. of dog.	Per cent of reducing substance in blood.		Per cent of reducing substance in urine.	Urine excreted per minute in cubic centimeters. <sup>1</sup>	Remarks.
		Before.	After.			
25	14.6	0.200	1 hr. 55 min. — 0.231	Minute trace.	0.13 0.10	Oxygen; clamp not completely applied and fall in b. p. only 20 mm. Hg.; clamp kept continuously on.
26	20.0	0.120	65 min. — 0.143	....	....	Oxygen; clamp applied for short periods; fall in b. p. marked.
27	95.0	0.197	80 min. — { 0.223 0.228	50 min. — 2.5 80 min. — considerable amount.	0.07 0.05	Oxygen; first urine collected 15 min. after experiment contained sugar, so that bladder urine probably contained it; reaction on b. p. of clamping was marked.
46	9.05	0.211 (After hepatic artery tied and tissues round p. v. dissected out.)	45 min. — 0.352 105 min. — 0.373	Small amount. Distinct.	0.10 ....	Fed with flesh and sugar day before; O <sub>2</sub> freely given; tissues around p. v. ligated but not cut; reaction on b. p. marked.
48	12.2	0.234	60 min. — 0.350 90 min. — 0.404	Absent.	0.13	Fed with large amount of flesh and sugar day before; O <sub>2</sub> administered; clamp <sup>1</sup> for 1 min. every two minutes.

<sup>1</sup> The time periods of collection of samples are given in corresponding line of preceding column.

the exact conditions in the two observations a little more closely, we shall see that they are by no means parallel. In the above experiments the portal circulation is suddenly stopped for some minutes and then restored, whereas in Eck's operation it is permanently shut off. Admitting for the present that in both cases hyperglycogenolysis is set up by the block, then the product of this (*i. e.*, sugar) will be suddenly washed into the systemic circulation when the portal circulation is restored; whereas when no restoration occurs it will only be slowly carried into the systemic circulation and will not therefore overwhelm the blood with sugar. In the one case, glycolysis can keep pace with the sugar production; in the other, it cannot.

Disturbances with the portal circulation in man are not associated with glycosuria. Cases are recorded in which there has been complete thrombosis of the portal vein without any glycosuria.

The next two types of experiments were performed with the object of showing that it is by direct action on the liver itself, and not indirectly through changes in the splanchnic circulation or by afferent stimulation of the medulla, that the hyperglycogenolysis is produced.

The amount of sugar in the blood as influenced by stimulation of the greater splanchnic nerve after cutting the hepatic nerves.

All the tissues running to the hilus of the liver except the portal vein were cut between mass ligatures. In this way all branches of the coeliac plexus proceeding to the liver were severed. The ligation of the hepatic arteries necessarily involved by this method has been shown above not to have any influence on the blood sugar content. The outer coat of the portal vein was cleaned as far as possible. The other details of the experimental procedure were as described on page 375. Examination of the seat of operation after death showed that in the last two cases reported all the tissue around the portal vein had not been cut.

Of the four experiments of this nature performed, no one showed any marked increase of blood sugar; although in No. 30 the maximal normal value for this was somewhat overstepped after ninety minutes' stimulation. The urine likewise remained practically free of reducing substance and there was no diuresis.

In these experiments, the splanchnic blood vessels were still constricted by the stimulation and the usual rise in arterial blood pressure was obtained. The only thing different in these as compared

TABLE V.  
CUT NERVES TO LIVER AND STIMULATE GREAT SPLANCHNIC NERVE.

No. of expt.	Wt. of dog.	Per cent of reducing substance in blood.		Per cent of reducing substance in urine.	Urine excreted per minute in cubic centimeters.	Remarks.
		Before.	After.			
28	18.0	0.162 (After operations.)	..... 90 min. — 0.189	50 min. — no trace. 90 min. — 0.85	0.1 0.2	Oxygen; rise of b. p. on stimulation of splanchnic not marked; b. p. 90 mm.; breathing affected by stimulation. <i>post mortem</i> nerves all cut.
30	6.3	0.191 (After operations.)	40 min. — 0.162 90 min. — 0.229	40 min. — free. ....	0.08 ....	Oxygen; <i>post mortem</i> record not given. Good b. p. with marked rise when splanchnic stimulated.
31	9.5	0.171 (After operations.)	55 min. — 0.184	....	....	Oxygen very slowly; b. p. greatly affected by technique but gradually recovered; marked rise on stimulation of splanchnic; <i>post mortem</i> considerable tissue left; $MgSO_4$ got into circulation 30 min. previous to last blood specimen.
32	9.0	0.184 (After operations.)	50 min. — 0.183 110 min. — 0.164	.... ....	.... ....	Oxygen; rise in b. p. not very marked on stimulation; <i>post mortem</i> considerable tissue left around portal vein.

with those experiments recorded in Table I was that the nerve impulse could not travel into the liver. We may conclude, therefore, that changes in the *extrahepatic* blood pressure do not cause hyperglycogenolysis provided that no asphyxial condition exists. Before considering the value of these experiments as bearing on the question under discussion we will proceed with the results of the next series.

The blood sugar content as influenced by stimulation of the hepatic nerves.

Instead of attempting to isolate the hepatic nerve plexus from the tissues in which it lies, the tissue was laid on the two wires of a pair of electrodes and these bent round so as to include all the nerve fibres. By such application of the electrodes it is of course impossible to be certain that most of the electrical current is not short-circuited through other tissue than nerves, but it was thought better to adopt such a technique rather than attempt a dissection of the plexus.

The results of these experiments were not entirely satisfactory, negative results having been obtained in Nos. 37 and 40; and in one of the remaining experiments, *viz.*, No. 35, although positive, the results are not conclusive, since the electrodes evidently became displaced and caused tetanus of the diaphragm during the passage of the current. In two of the experiments, Nos. 33 and 38, however, distinct hyperglycæmia was caused by the stimulation; and although quantitative estimations of the reducing power of the urine were not made, yet, by qualitative tests, glycosuria was found to be present in both cases. The carotid blood pressure did not show any rise in No. 33. In No. 38 there was a rise of a few millimetres Hg. early in the experiment, but later this was not seen.

Although, as discussed above, the hepatic nerves undoubtedly convey vaso-constrictor impulses to the intrahepatic portion of the portal vein, yet these do not in every case appear to act strongly enough to cause any constant change in the arterial blood pressure. This observation stands in agreement with similar ones by Mall,<sup>17</sup> who in six observations of exactly the same nature as those here recorded found no change in carotid blood pressure in one, and only a rise of a few millimetres in the others, and in these the rise was delayed for at least 20 seconds.

The results of these two groups of experiments recorded in Tables

<sup>17</sup> MALL: *Loc. cit.*, Versuche vi, vii, viii.

TABLE VI.  
STIMULATION OF PERIPHERAL ENDS OF HEPATIC NERVES.

No. of expt.	Wt. of dog.	Per cent of reducing substance in blood.		Per cent of reducing substance in urine.	Urine excreted per minute in cubic centimeters.	Remarks.
		Before.	After.			
35	5.0	0.103	50 min. — 0.231 95 min. — 0.231	....	....	Oxygen; b. p. at first markedly rose on stimulation and respiration deep and quick; after some time the b. p. fell instead of rising; the diaphragm was thrown into tetanus by stimulation; P. M. revealed much tissue around which electrodes were not placed.
33	8.5	....	60 min. — 0.325 90 min. — 0.360	30 min. — 0.5 90 min. — 0.5	0.66 0.40	Oxygen; b. p. somewhat low, 90 mm. Hg.; stimulation had no effect on b. p.
37	12.6	....	30 min. — 0.145 90 min. { 0.145 { 0.151	30 min. { mere { trace. 60 min. { trace.	0.33 0.43	Oxygen; b. p. at first rose on stimulation but later fell; <i>post mortem</i> showed that electrodes not placed around nerves.
38	8.9	0.210 (After all operations.)	60 min. — 0.264 120 min. — 0.303	30 min. — distinct. 60 min. — lost. 120 min. — trace.	Lost. .... Few drops.	Oxygen; stimulation no effect on b. p. or respiration. Just before last specimen of blood taken MgSO <sub>4</sub> got into circulation and artificial respiration necessary; <i>post mortem</i> electrodes properly applied.
40	11.4	0.118 (After all operations.)	60 min. — 0.117 90 min. — 0.099	60 min. — trace. 90 min. { consid- { erable. 120 min. { large { amount.	0.35 0.27 0.13	Oxygen; electrodes applied to ligatured pedicle by hand; quantitative sugars not done on urine but careful comparative tests made.

V and VI cannot of course be taken as absolute proof of the existence of glycogenase secretory fibres in the greater splanchnic nerves; but they are of value in the present discussion, inasmuch as they show us, first, that glycogenase formation is not brought about by the sudden changes in portal blood pressure induced by vasoconstriction in the splanchnic area; and secondly, that the nerve impulses (whether secretory or vaso-motor) which do have this effect are carried into the liver by the hepatic nerves and act *locally*. The fact that the two experiments of Table VI, in which the most marked hyperglycæmia was obtained as a result of stimulation of the hepatic nerves, were those showing no marked effect on general blood pressure, stands in accord with the view that specific secretory fibres are contained in these nerves.

*The blood sugar content as affected by splanchnic stimulation after the administration of atropin sulphate.*

In case splanchnic stimulation should not be followed by hyperglycæmia in atropinized dogs, it could be inferred that the glycogenase secretory nerve terminations had been paralyzed; on the other hand, should hyperglycæmia still follow stimulation, no conclusion one way or the other could be drawn from the result, for the liberation of glycogenase in the liver can scarcely be considered as an analogous process to the secretion of a digestive fluid, or of sweat or tears, on which atropin has a paralyzing action. Indeed, glycogenase formation is more closely allied to that of an internal secretion, such as is supposed to be produced by ductless glands, than it is to the above-mentioned secretory mechanisms, and we have no reason for believing that internal secretions are influenced by atropin.

Table VII gives the results of the experiments so far performed in this connection. It was found, even with liberal administration of oxygen, that atropin (1 mg. per kilo body weight) itself causes more or less hyperglycæmia (No. 57); so that the hyperglycæmia observed in atropinized dogs in which the splanchnic nerve was stimulated (Nos. 15, 17, 18, 59) does not offer us any assistance in connection with the above question. It is, however, considered wise to place these results on record, since it has been stated that stimulation of the celiac plexus does not cause hyperglycæmia after atropin.<sup>18</sup>

<sup>18</sup> CUSHNY, A. R.: A textbook of pharmacology and therapeutics, New York, 1899, p. 275. I have been unable to trace the origin of this statement.

TABLE VII.  
THE EFFECT OF SUBCUTANEOUS INJECTION OF ATROPIN SULPHATE (1-1.5 MG. PER KG. BODY WT.). STIMULATION OF THE SPLANCHNIC NERVE IN ATROPINIZED DOGS.

No. of expt.	Wt. of dog.	Per cent of reducing substance in blood.		Per cent of reducing substance in urine.	Urine excreted per minute in cubic centimeters.	Remarks.
		Before.	After.			
16	12.0	0.196	..... 120 min. — 0.259	60 min. — 3.3 120 min. — 3.8	0.10 0.19	No oxygen; not specially fed; atropin sulph. 1 mg. per kg. subcut.; pressor effect on stimulation of uncut vagus.
57	7.1	0.195	60 min. — 0.227 135 min. — 0.219	60 min. — 4.0 135 min. — 7.5	0.13 0.05	Oxygen; fed with flesh; atropin sulph. 1 mg. per kg. subcut.; Pressor effect on stimulation of uncut vagus.
58	5.7	....	65 min. — 0.335	65 min. — 0.9	0.03	Oxygen; fed with flesh; atropin sulph. 1 mg. per kg. subcut. after second blood removed.
59	....	0.179	60 min. after injection. 0.223 Spl. then stimulated. 40 min. after 0.265	60 min. — 9.0 30 min. — 9.0 40 min. — 8.3	0.2 0.26 0.24	Oxygen; no food for 48 hrs. previous to experiment; artif. resp. bellows used two hours after start of experiment.
15	6.6	....	After stimulation. 10 min. — 0.277 70 min. — 0.407 160 min. — 0.419	67 min. — 1.56 160 min. — 1.9	0.02 Few drops.	Oxygen; effect of splanchnic stimulation after 1 mg. per kg. atropin sulph.; artificial resp. bellows used two hours after start of experiment; pressor effect on stimulation of vagus.
17	10.5	0.191	63 min. — 0.214	50 min. — none.	0.09	Oxygen; artificial respiration; atropin sulph. 1.4 mg. per kg.; then splanchnic stimulated.
18	....	....	60 min. — 0.259 120 min. — 0.256	60 min. — 0.65 120 min. — 1.5	0.25 0.11	Oxygen and artificial respiration; atropin sulph. 1 mg. per kg.; splanchnic stimulation.



It is of interest further to note that the free administration of oxygen by the method described above does not prevent atropin hyperglycæmia and glycosuria. In this respect the action of atropin differs from that of such drugs as curare, ether, carbon monoxide, piperidin, nicotin, coniin, morphine, etc., which cause hyperglycæmia as a result of their action on the respiratory centre, producing a tendency to dyspnœa,<sup>19</sup> but which do not cause these results when oxygen is freely administered. In several of the experiments in which atropin was employed, a most striking glycosuria was observed without any very marked hyperglycæmia. Thus in No. 59 the percentage of reducing substance in the urine rose to nine in the sample collected during the sixty minutes immediately following injection of the drug, and it did not rise higher than this in the next forty minutes during which the great splanchnic nerve was stimulated. On the other hand, the percentage of reducing substance in the blood did not rise to anything like a corresponding degree. Much the same state of affairs is seen in Nos. 57 and 16. In the other experiments of this group, marked hyperglycæmias were noted in two, in one of which (No. 58) the splanchnic nerve was not stimulated, whereas, in the other (No. 15) it was. In neither of these cases was there any noteworthy glycosuria or diuresis.

It would appear from these observations that under certain conditions atropin may have a phloridzin-like effect on the kidney, *i. e.*, it may increase the permeability of the renal filter towards sugar. Further investigations will, however, be necessary before any definite statement can be made in this connection.

#### RÉSUMÉ.

In the present communication are recorded further observations (on dogs) dealing with the effect of stimulation of the great splanchnic nerve (left) on the per cent of reducing substance in the blood. The results corroborate those of the first communication, *viz.*, that a more or less marked hyperglycæmia becomes established within half an hour.

The amount of urine excreted and the percentage of reducing substance in the urine usually become increased to a corresponding degree.

<sup>19</sup> UNDERHILL: *Loc. cit.*

When the stimulus is maintained for several hours (off and on), the hyperglycæmia reaches a maximum, after which it declines, this being also the case with the diuresis and glycosuria. The exact time (after commencement of stimulation) of this maximum probably varies in different animals, being in about two hours in the experiments so far performed.

A number of experiments are then offered bearing on the question of the exact mechanism by which stimulation of the splanchnic nerve leads to the above results. The possibilities considered are:—

1. Afferent stimulation of the medullary centres.
2. Vasomotor changes in the liver.
3. Stimulation of secretory fibres controlling the production of glycogenase in the liver.

A final conclusion is deferred until after the third and fourth communications of this series are completed; but, so far, the following facts bearing on the question have been established:—

1. Liberal intrapulmonic administration of oxygen does not prevent the hyperglycæmia, although it may somewhat diminish it.
2. Stimulation of the cut hepatic nerves (peripheral ends) is followed by hyperglycæmia.
3. Stimulation of the great splanchnic nerve after cutting the hepatic nerves is not followed by hyperglycæmia.
4. Ligation of the hepatic artery is not followed by hyperglycæmia.
5. Clamping of the portal vein for short periods of time (less than one minute) is not followed by hyperglycæmia.
6. Atropin (1 mg. per kilo body weight subcutaneously) does not prevent the hyperglycæmia which follows stimulation of the great splanchnic nerve.

My thanks are due to Mr. R. H. Waters for his valuable assistance in the conduction of the chemical analyses.

STUDIES IN EXPERIMENTAL GLYCOSURIA. — NO. III.  
THE INFLUENCE OF STIMULATION OF THE  
GREAT SPLANCHNIC NERVE ON THE RATE OF  
DISAPPEARANCE OF GLYCOGEN FROM THE  
LIVER, DEPRIVED OF ITS PORTAL BLOOD SUP-  
PLY OR OF BOTH ITS PORTAL AND SYSTEMIC  
BLOOD SUPPLIES.

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IN the second of this series of papers on experimental glycosuria it was shown that very considerable changes in the blood supply to the liver — such as those produced by ligation of the hepatic artery or occasional clamping of the portal vein — do not tend to a hyperglycæmic state. It was pointed out that these observations are of importance in elucidating the mechanism of the hyperglycæmia which follows stimulation of the great splanchnic nerve, since this hyperglycæmia might possibly be accounted for by the local anæmia in the liver brought about by stimulation of vaso-constrictor fibres. The rapid glycogenolysis which sets in immediately after death is more probably the result of vascular stagnation in the hepatic vessels than of a severance of nerve connection (Claude Bernard), so that it must always be considered as at least a possibility that a less marked diminution in blood supply, such as would result from vaso-constriction of the portal vein or hepatic artery during life, might bring about a similar result. The experiments referred to above would indicate that such an explanation is improbable.

Having further shown that afferent stimulation of the medullary centres cannot be accepted as an explanation of splanchnic hyperglycæmia, we are driven by exclusion to the hypothesis that the splanchnic nerve contains fibres which regulate the production (or activity) of glycogenase in the liver; in other words, of glycogenase-secretory fibres. In the present communication an attempt is made

to furnish direct proof of the secretory nerve hypothesis, by comparing the rate of disappearance of glycogen in pieces of liver removed during stimulation of the splanchnic nerve with that found in pieces removed when there is no such stimulation.

In one series of experiments the portal vein was anastomosed with the vena cava, a piece of liver removed for glycogen determination, the animal left undisturbed for one hour and then another piece removed. The rate of glycogenolysis in these cases is compared with the rate found in other animals in which, during the hour's interval between removal of the two pieces of liver, the splanchnic nerve was stimulated.

In another series of experiments, besides making an anastomosis between the vena porta and the vena cava, the hepatic artery was ligated; a piece of liver was then removed for glycogen estimation, ten minutes allowed to elapse, another piece of liver removed, after which the splanchnic nerve was stimulated for a further ten minutes and the glycogen determined to a third piece of liver. The rate of glycogenolysis during the first is compared with that occurring during the second period of ten minutes.

Another series of experiments of a somewhat similar nature was attempted on the liver of the (snapping) turtle.<sup>1</sup> In this animal the liver consists of two lobes joined together by a narrow bridge of liver tissue. By ligaturing this connecting bridge of tissue with a flat ligature (shoe lace) one lobe can be removed without any loss of blood. An hour was allowed to elapse between removal of the two lobes during which time, in the case of some of the animals, the spinal cord was stimulated electrically, whilst in others the animal was left undisturbed. The percentage of glycogen was determined in the two lobes. It was hoped that a comparison of the rate of disappearance of glycogen in the stimulated and non-stimulated cases would show a more rapid glycogenolysis as a result of stimulation. It was found, however, that very irregular results were obtained; so much so that it will be necessary to repeat the experiments and report the results in some further communication.

Experiments somewhat similar in type to the above were performed by Cavazzani<sup>2</sup> to show that fibres exist in the coeliac plexus which control the disappearance of glycogen from the liver. This worker did not, however, attempt to keep his animal alive but

<sup>1</sup> GRUBE KARL: *Archiv für die gesammte Physiologie*, 1907, cxviii, p. 1.

<sup>2</sup> CAVAZZANI: *Ibid.*, 1894, lvii, p. 181.

merely observed the rate of *post mortem* glycogenolysis of the liver *in situ* during stimulation of the coeliac plexus. He found that stimulation for from five to ten minutes caused more than half of the original glycogen to disappear from the liver, and concluded that so rapid a disappearance could be explained only on the basis that secretory fibres exist in the coeliac plexus. He also examined under the microscope the appearance of sections of liver before and after stimulation of the plexus, and found that shrinkage of the hepatic cell and diminution in stainability towards iodine resulted from stimulation. In the publication on his work which I have at hand, no data are given of the methods employed for estimation of glycogen, nor are protocols or tables of results recorded, so that it is impossible to estimate the value of Cavazzani's experiments. Nothing is stated regarding the rate of glycogenolysis immediately after death without any stimulation of the splanchnic nerve.

In order to keep up the stimulation for longer periods of time than those employed by Cavazzani, the animals were not killed in the present research, but by establishing the Eck fistula the portal blood was prevented from traversing the liver. In this way also the circulation through the coeliac plexus is left intact, thus obviating any block to the nerve impulse due to anæmia in the ganglia. The importance of keeping intact the nerve connections between the liver and central nervous system lies in the fact that *post mortem* glycogenolysis has been claimed to be due to removal of nervous control (Bernard). The temperature of the liver is also kept more constant by such a procedure.

THE RATE OF GLYCOGENOLYSIS IN ONE HOUR IN THE LIVER AFTER  
ANASTOMOSING THE VENA PORTA TO THE VENA CAVA, WITH  
AND WITHOUT STIMULATION OF THE GREAT SPLANCHNIC  
NERVE (LEFT).

It has already been shown that ligation of the hepatic artery does not have any effect on the percentage of blood sugar.<sup>3</sup> It was thought that under such circumstances it would be permissible to leave the circulation through the liver by way of these vessels intact and yet to assume, did glycogen disappear from the liver more rapidly as a result of stimulation of the splanchnic nerve than it

<sup>3</sup> MACLEOD: This journal, 1908, xxii, p. 374.

would without such stimulation, that the mechanism involved could not be a change in the blood supply. In accepting this argument, however, it must be remembered that it has not yet been shown that ligation of the hepatic artery has no effect on glycogenolysis in the liver when the portal blood supply is also cut off. By leaving the circulation through the hepatic artery undisturbed, the oxygen supply to the hepatic lobule will be maintained, for, as pointed out in the previous article, little of this supply can be considered as being derived from the portal blood. Therefore, although a comparison of the rate of glycogenolysis in one hour, during which the splanchnic nerve is stimulated with that occurring when there is no nerve stimulation, must evidently be of importance in connection with the question at issue, yet the value of the result must not be overestimated at the present; since it is conceivable that the branches of the celiac artery become nearly obliterated when the splanchnic nerve is stimulated, thus causing the circulation to the liver lobule and celiac plexus to become almost entirely cut off so that a virtual *post mortem* glycogenolysis sets in.

*Methods.*—The anastomosis between the vena porta and vena cava was effected by means of a small brass tube 5 mm. long with an internal diameter of 6 mm., and having on its outer side two grooves. A small brass tongue bent at right angles to the tube projects from one end so as to enable the tube being held firmly in a hæmostat.<sup>4</sup> The portal vein is cleaned of surrounding tissue and the pancreatico-duodenal branch ligated at its junction with the portal vein. The vena cava between the renal veins and the liver is similarly cleaned and loosened from its connections. The portal vein is then ligated as far up as possible (*i. e.*, just before it divides preparatory to entering the liver) and a hæmostat with its blades covered with india rubber tubing is applied to the vein about 1 inch lower down, after which the vein is cut across just below the ligature. The transfusion cannula is then laid over the cut vein with its free border above, and the vein caught hold of in a pair of artery forceps with fine blades and pulled through the cannula. Two more artery forceps are then applied to the cut edge of the vein, by means of which the vein is folded over the cannula and a ligature applied in the lower groove (*i. e.*, in the groove next the holding strip). The vena cava is next clamped near the liver with a protected pair of hæmostats and a ligature

<sup>4</sup> A cannula of similar construction is employed by Dr. G. W. CRILE.

is applied just above the renal veins, after which the vein is cut across just above the ligature and the edges caught up by means of the fine hæmostats. It is now an easy matter to insert the portal vein cannula into the vena cava and to tie it in by means of a ligature applied to the upper groove. The above operation occupies, with practice, about ten minutes and, since the intima of the two vessels is brought in contact, little danger of clotting is incurred.

The arterial blood pressure after the anastomosis is established is usually somewhat below the normal; in some cases where the operation has not been skillfully performed it is quite low, 40–60 mm. Hg.

A piece of liver (sometimes from two lobes) was then removed, hæmorrhage being prevented by applying a mass ligature, and its glycogen content determined by the method of Nerking-Pflüger. In certain of the observations the dog was left undisturbed for one hour; in others the great splanchnic nerve on the left side was stimulated electrically for a part or the whole of this period. A second piece of liver was then removed for glycogen estimation. A comparison of the percentage of glycogen found in the first and second portions of liver furnishes evidence of the rate of glycogenolysis.

In the above experimental procedure it is assumed that the percentage of glycogen in different parts of the liver is approximately equal, although there appears to be some diversity of opinion regarding the point. Pflüger,<sup>5</sup> mainly on the basis of observations made by Karl Grube,<sup>6</sup> concludes that the percentage amounts of glycogen of different portions of the liver of well fed dogs do not show a difference from one another of more than 5 per cent. This conclusion is in harmony with that of Seegen and Kratschmer,<sup>7</sup> who extracted the glycogen by frequent boiling with water, and with those of Richard Külz<sup>8</sup> and Cramer,<sup>9</sup> who employed the Brücke-Külz method for estimating the glycogen. It is at variance with the still older results of Von Wittich.<sup>10</sup> Abderhalden and Rona state that glycogen is not equally distributed throughout the liver, although they give no experimental evidence for their statement.

<sup>5</sup> PFLÜGER: *Das Glykogen*, Bonn, 1905.

<sup>6</sup> GRUBE: *Archiv für die gesammte Physiologie*, 1905, cvii, p. 483.

<sup>7</sup> SEEGEN and KRATSCHMER: *Archiv für die gesammte Physiologie*, 1880, xxii, p. 223.

<sup>8</sup> RICHARD KÜLZ: *Zeitschrift für Biologie*, 1886, xxii, p. 183.

<sup>9</sup> CRAMER: *Zeitschrift für Biologie*, 1888, xxiv, p. 85.

<sup>10</sup> VON WITTICH: *Cf.* SEEGEN and KRATSCHMER's article, *loc. cit.*

TABLE I.  
THE PERCENTAGE DISAPPEARANCE OF GLYCOGEN FROM THE LIVER IN ONE HOUR AFTER THE ESTABLISHMENT OF THE ECK FISTULA,  
WITH AND WITHOUT STIMULATION OF THE SPLANCHNIC NERVE.

Number and nature of experiment.	Per cent glycogen (dext.) in piece of liver removed immediately after establishing fistula.	Per cent glycogen (dextrose) in piece of liver removed one hour later.	Loss of glycogen (dextrose) in one hour.	Loss of glycogen (dextrose) in per cent of original glycogen.	Per cent reducing substance in blood.	Remarks.
No Stimulation.						
2. Eck fistula.	2.543	0.657	1.886	74.1	0.317 after 1 hour	Operation quickly performed. B. P. low throughout. (/vis mortem clot in portal vein. Intestines cyanotic.)
3. Eck fistula.	1.645	1.244	0.401	24.3	0.331 after 1 hour	Operation quickly performed. B. P. good throughout. (Fed with flesh and sugar.)
7. Eck fistula.	1.071	0.643	0.428	40.0	0.122	Op. perf'd with difficulty. B. P. 40-45 mm. Hg. (Fed with flesh and sugar.)
8. Eck fistula.	2.130	1.647	0.483	22.6	....	Operation quickly perf'd. B. P. 85 mm. Hg. (Fed with flesh and sugar.)
4. Eck fistula and intermittent stimulation of splanchnic.	2.703	1.638	1.065	39.4	0.148 after 1 hour	Operation quickly performed. B. P. 65 mm. rising to 110 on stim. of splanchnic. (Fed with flesh and sugar.)
5. Eck fistula, splanchnic stimulated for first 10 minutes of hour period.	1.777	0.436	1.241	70.0	0.090	The splanchnic nerve found divided into branches in supra-renal region. Only one of these stim., with little effect on B. P. (Fed with flesh and sugar.)
6. Eck fist. Spl'nic continuously stim. strong current.	0.730	none	0.730	100.0	0.146 after 1 hour	B. P. 65 rising to 90 on stimulation and gradually falling to 75 mm. (Fed with flesh and sugar.)
9. Eck fistula. Branch of splanchnic stimulated continuously.	0.437	0.047	0.390	89.2	0.238 after 1 hour	B. P. 50 mm. rising only slightly on stimulating. (Fed with flesh and sugar.)
10. Eck fistula. Splanchnic stimulated for 40 minutes.	1.607	0.271	1.336	83.1	0.216	B. P. 50 rising to 90 mm. on stimulating. Breathing stopped 10 min. after start of stim. (arti. resp.) recovery. (Fed with flesh and sugar.)
11. Eck fistula. Splanchnic stim. for 30 m. beginning 30 m. after making fistula.	2.269	1.116	1.153	50.8 (in 30 min.)	....	B. P. 75 mm. Hg. rising to 115 mm. on stimulating. (Fed with flesh and sugar.)
Splanchnic Nerve Stimulated.						



Sérégé,<sup>11</sup> using Fränkel's method, found the right and left portions of the liver to contain different amounts of glycogen.

From the table it will be seen that there were four "resting" experiments. In two of these (viz., No. 3 and No. 8) only about 25 per cent of the glycogen present at the beginning disappeared in the hour; in a third case, 40 per cent disappeared (No. 7), and in the remaining one, 75 per cent (viz., No. 2).

A most important question presents itself in connection with these results: why does diversion of the portal blood into the systemic circulation cause so rapid a glycogenolysis? It was noted by Claude Bernard and others that there was a rapid accumulation of reducing substance in the liver immediately after death, and that later the amount grew more slowly. It was suggested by Bernard that this accumulation is partly the result of the stoppage of the blood flow, the sugar not being carried away into the circulation; and partly because the normal glycogenolytic power of the liver becomes more active when the influence of the nervous system has been removed.<sup>12</sup> Bernard quotes experiments by Dalton, who found that in the liver of the rabbit the percentage of dextrose was 2.675 in four seconds after death; 11.358 in one hour; 13.861 in four hours, and 15.361 in twelve hours. Pavy also noted that in a few minutes after death the percentage of reducing substance in the liver had risen from somewhere between 0.1 and 0.4 to 1.2 or 1.5 per cent, and in twenty-four hours to from 2 to 3.5 per cent. Seegen and Kratschmer<sup>13</sup> also record several observations on the rate of accumulation of sugar in, and the rate of disappearance of glycogen from the liver after death, but the results are of little value in connection with the present research. Pavy argued that the *post mortem* glycogenolysis is due to the development in the liver of a ferment (glycogenase) and that it is not a continuance of an *ante mortem* process. The results obtained in the present research show that hepatic glycogenolysis becomes exaggerated in proportion to the extent to which the circulation through the viscus is disturbed, and there is nothing in them which would suggest that removal of nervous control had anything to do with the incidence of this process. Glycogenolysis is produced by diversion of the portal blood alone — the arterial blood supply being undisturbed — but it becomes very

<sup>11</sup> SÉRÉGÉ: Cf. PFLÜGER, Das Glykogen, 1905, p. 149.

<sup>12</sup> CLAUDE BERNARD: Leçons sur le diabète, 1877, p. 351.

<sup>13</sup> Loc. cit.

much more marked when, as well as absence of portal blood, there is a diminution of the arterial blood supply. Such diminution of arterial blood supply is seen in Experiment No. 7, and still more so in Experiment No. 2. In both of these cases the pressure of the carotid artery was very low in contrast to that observed in the other two experiments (No. 3 and No. 7).

That absence of the portal circulation alone causes a more rapid glycogenolysis, even when the hepatic artery circulation is maintained, is further confirmed by the results reported in the previous communication on the increase in percentage of reducing substance in the blood following prolonged clamping of the portal vein.

*We must conclude, therefore, that absence of the portal blood supply stimulates a glycogenolysis which becomes much more marked when there is also a deficient hepatic artery supply.*

Coming now to the results obtained when the splanchnic nerve was stimulated. In all there were six experiments of this nature. In the first two of these (No. 4 and No. 5) the glycogenolysis in one hour was no greater than that observed with no stimulation and a poor arterial blood pressure, but in the remaining four (Nos. 6, 9, 10, and 11) the glycogenolysis was distinctly more rapid. At first sight such a result would seem to confirm the belief that specific secretory fibres had been stimulated in the splanchnic nerve; but yet it is not unequivocal evidence of the truth of such an hypothesis, for it is quite conceivable that the vaso-constriction of the hepatic artery as a result of stimulation of the splanchnic nerve is so marked as to almost cut off the blood supply through this vessel, indeed, to produce a more nearly absolute anæmia than that consequent upon a low general arterial blood pressure. It should further be pointed out, in connection with these "stimulation" experiments, that in two of them (No. 6 and No. 9) there was a very small amount of glycogen in the liver to start with and that therefore the *absolute* amount of glycogen which disappeared was small though the *percentage* amount was high.

*As a result of these experiments, we must conclude that although they agree with the observations on the percentage of reducing substance in the blood in showing that stimulation of the great splanchnic nerve causes increased activity of the glycogenolytic function of the liver, yet they do not furnish any certain evidence that the fibres thus stimulated are secretory in nature; they might just as well be vaso-constrictor fibres.*

THE RATE OF GLYCOGENOLYSIS IN THE LIVER DURING ABSENCE OF BOTH PORTAL VEIN AND HEPATIC ARTERY BLOOD SUPPLY, WITH AND WITHOUT STIMULATION OF THE SPLANCHNIC NERVE.

The technique of these experiments involved the anastomosis of the vena porta to the vena cava, followed by ligation of the hepatic artery after most carefully isolating it from its accompanying nerve fibres. By these operations the liver, although deprived of both the portal vein and hepatic artery blood supplies, was yet in normal connection with the nervous system; it was also rendered practically bloodless except for back flow from the hepatic veins, so that, on cutting it, there was only slight hæmorrhage, which was soon arrested by clotting. A piece of liver from three different lobes was removed for estimation of glycogen, the animal was left undisturbed for ten minutes when a second piece of liver, also from three lobes, was removed. In three of the experiments another ten minutes was allowed to elapse when a third mixed sample of liver was removed. In another three experiments the splanchnic nerve and the hepatic branches of the celiac plexus were stimulated electrically during the second period of ten minutes, after which the third sample of liver was taken. The samples of liver for glycogen analysis were in these experiments removed from three lobes so as to diminish any possible error which might be incurred should the distribution of the substance not be quite equal over the viscus. The fact that no mass ligature had to be applied made this possible. There is still one other error which may have been incurred in the above technique, and that is that the last pieces of liver removed (from the centre of the viscus) may have contained relatively more connective tissue than the pieces first removed (from the edges). It would have been better to remove the samples in wedge-shaped portions.

An examination of Table II will show that the results of the three experiments in which no nerve was stimulated are very inconstant. Indeed, one of these (*viz.*, No. 76), is evidently quite incorrect, for according to it there was as much glycogen remaining in the liver after twenty minutes as after ten minutes. In another experiment (No. 69) the same amount of glycogen disappeared during both periods, and in the remaining experiment of the three

TABLE II.  
RATE OF GLYCOGENOLYSIS AFTER CUTTING OFF BLOOD SUPPLY TO THE LIVER, FOR TEN MINUTES BEFORE AND DURING STIMULATION OF THE GREAT SPLANCHNIC NERVE.

No.	Weight.	A. First ten minutes.				B. Second ten minutes.		
		Per cent glyco- gen in liver im- mediately after tying vessels.	Per cent gly- cogen 10 min- utes later.	Percentage amount of glycogen dis- appeared.	Amount gly- cogen disap- peared in per cent of origi- nal amount.	Per cent gly- cogen 10 min- utes later.	Percentage amount gly- cogen disap- peared.	Amount gly- cogen disap- peared in per cent of origi- nal amount.
69	12.65	1.629	1.391	0.238	14.61	1.139	0.252	15.45
76	4.4	0.889	0.536	0.353	39.5	0.574	+0.038	....
77	10.5	1.280	0.820	0.460	36.0	0.700	0.120	9.37
71	4.45	3.627	3.334	0.293	8.07	1.801	1.533	42.2
74	6.25	2.278	2.058	0.220	9.65	1.704	0.354	15.5
75	6.0	2.150	1.536	0.614	28.55	0.948	0.588	27.3
72	4.45	2.301	1.890	0.411	17.86	....	....	....

SPLANCHNIC  
NERVE STIM.  
No NERVE  
STIMULATED.

(No. 77) much more disappeared during the first period than during the second. In none of these three experiments, however, did a greater amount of glycogen disappear during the second period than during the first.

The rate of glycogenolysis in the livers of different animals (in the absence of nerve stimulation) is seen from the results given both in this table and in Table I to be very variable. In ten minutes, in the case of three of the experiments, about one third of the original store of glycogen disappeared, whereas in the other two cases there was a loss of only about one tenth. It is impossible to explain at present the cause of these variable results. We are confident that it does not reside in the chemical technique for we have carefully tested the reliability of this in duplicate analyses shortly to be published. Two sources of error must, however, be borne in mind; one of these we have already discussed, *i. e.*, the variable distribution of glycogen over the viscus (see p. 403), and the other is the variable amount of blood in different portions of liver. This latter error we have tried to minimize by pressing out the pieces of liver on filter paper. Even allowing for these possible fallacies we must nevertheless conclude that the rate of glycogenolysis after cutting off the blood supply varies considerably in the livers of different animals.

Turning now to the effect of stimulation of the splanchnic and hepatic nerves on the rate of glycogenolysis, we see that in two of the experiments (Nos. 71 and 74) considerable acceleration of this occurred. In one of the experiments (No. 75), however, such a result was not obtained, there being as great a disappearance of glycogen during the first as during the second period. Even in the case of the two experiments giving positive results in this connection, there is some doubt as to whether the nerve stimulation was responsible, for it will be noted that during the first ten-minute periods in these experiments, a comparatively low rate of glycogenolysis was observed (*viz.*, 8.0 and 9.6 per cent), which may conceivably have been due to the fact that the liver had not reacted within this period to the deprivation of its blood supply; in other words, that the onset of glycogenolysis was delayed.

Before drawing any final conclusions from the experiments here recorded it is evident that we must be furnished with more reliable and extensive data regarding the course of *post mortem* glycogenolysis. An examination of the literature covering this process has

shown us that practically nothing is known regarding the time after death at which glycogenolysis attains its maximum activity, and whether this maximum appears always at the same time. Without this information observations such as those recorded cannot of course be depended upon for drawing final conclusions, although the results thus far obtained are undoubtedly suggestive of the presence of secretory fibres in the splanchnic nerve.

Besides the observations on *post mortem* glycogenolysis of Dalton, Pavy, and Seegen above quoted, numerous tests have been made of the rate of glycogenolysis in incubated samples of minced liver previously washed free of blood.<sup>14</sup> The results of these researches do not, however, help us in connection with the present question. As mentioned above, it was claimed by Claude Bernard that *post mortem* glycogenolysis owes its incidence to the isolation of the liver from the central nervous system. In other words, he seems to have believed that during life the nervous system exercises an inhibitory or retarding effect on hepatic glycogenolysis, so that when disconnected from nervous influence glycogenolysis will run riot, as it were, and *post mortem* glycogenolysis will set in. This explanation is highly improbable since we have found no evidence of hyperglycæmia to follow cutting of all the hepatic nerves: it is much more likely that interference with blood supply is the determining factor of *post mortem* glycogenolysis. The results obtained in the previous communication and in this one would so far bear out this supposition and in our next paper the question will be more fully gone into.

#### RÉSUMÉ.

A comparison is made of the rate of disappearance of glycogen from the liver — after diverting the portal blood into the inferior vena cava — during stimulation of the great splanchnic nerve (left) in one group of animals with that occurring in another group of animals without any such stimulation. The amount which disappeared in a given time (1 hour) is found to be greater in the group in which the nerve was stimulated. Such a result cannot be taken as final evidence of the presence of secretory glycogenolytic fibres in the great splanchnic nerve, because the constriction of the hepatic artery produced by the stimulation may have been sufficient to

<sup>14</sup> Cf. PICK, G. F.: HOFMEISTER'S Beiträge zur chemischen Physiologie und Pathologie, 1902, iii, p. 163.

render the liver almost bloodless, and thus to bring on *post mortem* glycogenolysis.

A comparison is also made between the rate of disappearance of glycogen from the liver deprived of both portal vein and hepatic artery blood supplies but with its nervous connections intact, for ten minutes before and for ten minutes during stimulation of the great splanchnic nerve. In two out of three experiments the disappearance was distinctly greater during the second or stimulation period. Provided that complete anæmia of the liver is always immediately followed by a glycogenolysis which progressively gets less in amount then the above result would furnish positive proof of the presence of secretory glycogenolytic fibres in the splanchnic nerve. Three experiments are recorded in which during both periods of ten minutes in a bloodless liver, without stimulation during either period, the rate of glycogenolysis, though variable, was the same or less during the second, as compared with the first period.









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## ON THE USE OF NITROUS ACID, NITRITES, AND AQUA REGIA IN THE DETERMINATION OF THE MINERAL CONSTITUENTS OF URINE.

By J. H. KASTLE.

[From the Division of Chemistry, Hygienic Laboratory, U. S. Public Health and Marine Hospital Service, Washington, D. C.]

THE complete incineration at low red heat of the residue left after the evaporation of urine is a tedious and discouraging operation. The difficulties encountered in this process are due partly to the presence of alkali phosphates and partly to the fact that urea itself is not as readily decomposable by heat as one might imagine. In this connection it has already been pointed out by Folin<sup>1</sup> that while urea is both unstable and volatile at high temperatures, it is by no means an easy task to burn pure urea completely into carbonic acid and ammonia. This author is of the opinion, therefore, that it is impossible to isolate the mineral constituents from human urine or from the urine of carnivorous animals by direct evaporation and ignition, and he is inclined to doubt whether more than a very small proportion of the potassium determinations now recorded in the literature are even approximately correct on account of contamination with ammonia.

My own attention was first attracted to this subject some time ago in connection with the examination of a sample of urine from a case of exophthalmic goitre, and it occurred to me in this connection that it might be advantageous to get rid of the urea and perhaps other amino compounds and readily oxidizable substances, by preliminary chemical treatment of some sort before attempting the incineration of the residue left after the evaporation of the urine.<sup>2</sup> A consideration of the substances which might be employed

<sup>1</sup> FOLIN: This journal, 1903, ix, p. 273.

<sup>2</sup> The idea that certain oxidizing mixtures could be employed to advantage in the determination of the mineral constituents of various animal tissues and secre-

to advantage for this purpose led me to believe that possibly nitrous acid and certain of the metallic nitrites could be used, since, as is well known, nitrous acid reacts upon urea at slightly elevated temperatures, giving rise to nitrogen, carbon dioxide, and water. In fact this reaction has already been employed by Campani<sup>3</sup> in the quantitative estimation of urea. So far as I know, however, no one has ever employed this reaction for the purpose of removing urea, prior to the incineration of urine residues, in the determination of the metallic elements in urine.

#### ON THE USE OF SODIUM NITRITE AS AN AID TO THE RAPID AND COMPLETE INCINERATION OF URINE RESIDUES.

Of all the substances thus far tried, sodium nitrite has given the most satisfactory results in the rapid and complete incineration of urine residues. In order to determine the effect of the preliminary nitrite treatment of urine on the incineration of such residues, the following experiments with sodium nitrite were carried out:<sup>4</sup>

Ten cubic centimetres of human urine were measured into a 100 c.c. platinum dish, and to this there were added 1 gm. of sodium nitrite and 15 c.c. of *n*/1 hydrochloric acid. The dish containing these substances was then placed on the steam bath and the contents evaporated to dryness. The decomposition of the urea is marked by a gentle and regular effervescence, which begins almost as soon as the acid is added and is soon completed at the temperature of the

ions has already occurred to other chemists. Thus NEUMANN (*Zeitschrift für physiologische Chemie*, 1902, xxxvii, pp. 115-142), has employed for this purpose a mixture of equal parts by volume of concentrated sulphuric and nitric acids. Obviously, however, the introduction into the solution to be analyzed of relatively large amounts of sulphuric acid is not without its disadvantages in certain determinations.

<sup>3</sup> CAMPANI: *Gazzetta chimica italiana*, 1887, xvii, p. 137.

<sup>4</sup> Sodium nitrite of a reasonable degree of purity is now supplied by manufacturers of pure chemicals. The so-called c. p. salt may be purified by recrystallization from water and may thus be obtained in excellent condition. A specimen of the recrystallized salt prepared by Mr. ELVOVE has been preserved in glass-stoppered bottles for some time in this laboratory in practically unaltered condition. During very wet weather the recrystallized salt has been found to be somewhat hygroscopic; otherwise it seems to undergo no alteration, and so far as we have been able to ascertain from observations on a pure specimen of sodium nitrite, it would seem that the recrystallized salt can be preserved indefinitely in glass-stoppered bottles, especially if kept in a calcium chloride desiccator. On repeated evaporation with hydrochloric acid, 0.5620 gm.  $\text{NaNO}_2$  gave 0.4741 gm.  $\text{NaCl}$ ; theory, 0.4761 gm.

bath. After evaporating to dryness, the dish was removed from the bath, covered with a platinum cover, and gradually heated to low red heat over a circular burner, one to two minutes being required for complete incineration.<sup>5</sup> Considering the extreme slowness of the combustion of urea residues, as such combustions are ordinarily carried out, the rapidity and completeness of the incineration at low red heat following the removal of urea by means of sodium nitrite and hydrochloric acid are in reality but little short of remarkable. The white residue left after the evaporation and incineration of 10 c.c. of urine, 1 gm. of sodium nitrite and 15 c.c. *n*/1 HCl, has been generally found to have an alkaline reaction and to contain small amounts of nitrites and carbonates. These are removed by the addition of a small amount of hydrochloric acid, water is then added, and the solution is now ready for the determination of the several metallic elements of the urine. These may be determined by the methods ordinarily employed in ash analysis.<sup>6</sup> Up to the present, this method has been utilized only in the determination of the alkali metals in urine.

In order to test the accuracy of the method as applied to the determination of sodium and potassium, a specimen of the writer's urine was collected and divided into two portions, one of which, No. 1, was used in its original form. To 40 c.c. of a second portion of the same urine, 0.1750 gm. of pure potassium chloride was added. This specimen was labelled No. 2. The sodium and potassium in each of these specimens was then determined by treating 10 c.c. of the urine with 1 gm. of sodium nitrite and 15 c.c. of *n*/1 HCl, evaporating to dryness, igniting at low red heat, dissolving the fused mass in water containing a small amount of hydrochloric acid, and removing the alkaline earths and phosphates by means of barium hydroxide,<sup>7</sup> precipitating the excess of barium with ammo-

<sup>5</sup> The time required for the complete incineration of the urine residues has been found to depend on the quantities of the urine and sodium nitrite employed, and also on the nature of the vessel in which the incineration is accomplished. On the supposition that urine contains two per cent of urea, 10 c.c. of urine would require 0.46 gm. of sodium nitrite and 6.6 c.c. *n*/1 HCl to effect the complete decomposition of the urea. With 10 c.c. of urine and quantities of sodium nitrite ranging from 0.25 to 1. gm., and 15 c.c. *n*/1 HCl, from 1 to 10 minutes have been required to effect the complete incineration of the residues, depending on whether platinum or porcelain dishes were employed.

<sup>6</sup> See SOLDNER: *Zeitschrift für Biologie*, 1902-1903, xliv, pp. 65-69.

<sup>7</sup> According to some authors, milk of lime is preferable to barium hydroxide for accomplishing the removal of the phosphates and magnesia.

nium carbonate, etc., evaporating the filtrate to dryness, igniting to drive off the ammonium salts and weighing the mixed chlorides of sodium and potassium, after which the potassium was determined as potassium chlorplatinate in the usual manner. The results of these determinations are given in Table I.

TABLE I.

No. of specimen.	Quantity of urine taken for analysis	Sodium nitrite added.	NaCl + KCl found.	Weight of $K_2PtCl_6$ .	Weight of KCl found.	Weight of KCl added to urine No. 2, found.	Weight of KCl actually added to urine No. 2	NaCl found.
1	10 c.c.	1 gm. <sup>1</sup>	1.0245	0.1232	0.0378	....	....	0.1395
2	10 c.c.	1 gm.	1.0695	0.2658	0.0816	0.0438	0.04375	0.1407
<sup>1</sup> Equivalent to 0.8472 gm. NaCl.								

(In this and the following tables all weights are in grams.)

With the object of still further testing the accuracy of this method, a specimen of the writer's urine, 1065 c.c., was collected during a period of 24 hours. With this specimen of urine the following solutions were prepared:

(1) 200 c.c. urine, + 50 c.c. *n*/1 HCl.

(2) 200 c.c. urine, + 50 c.c. *n*/1 HCl, + 0.2652 gm. KCl.

By way of further comparison a solution was prepared containing 200 c.c. of water, + 50 c.c. *n*/1 HCl, + 0.2652 gm. KCl. This was labelled (3). In these solutions the several amounts of sodium and potassium were determined after treatment with sodium nitrite and hydrochloric acid, evaporating to dryness and incinerating at low red heat. The results of these determinations are given in Table II.

It is evident, therefore, from the results of these determinations, that this method, involving the removal of urea from urine by means of sodium nitrite and hydrochloric acid prior to ignition, can be employed to advantage in the quantitative determination of potassium in urine and probably also in the determination of sodium. The only objections that might be urged against the method are, first, that relatively large amounts of chlorplatonic acid are required, involving also considerable washing with 80 per cent alcohol in the



separation of the chlorplatينات of potassium and sodium; second, as carried out in the manner above described, two ignitions are required; and third, the use of relatively large amounts of a somewhat hygroscopic sodium salt, such as the nitrite, obviously renders diffi-

TABLE II.

No. of specimen.	Quantity of urine or solution taken for analysis.	Sodium nitrite added. <sup>1</sup>	NaCl + KCl found.	Weight of $K_2PtCl_6$ .	Weight of KCl found.	Weight of KCl added to urine No. (2) and solution No. (3), found.	Weight of KCl actually added to urine No. (2) and solution No. (3).	NaCl found.
(1)	10 c.c.	0.5620	0.5843	0.0716	0.0220	....	....	0.0882
(2)	10 c.c.	0.5620	0.5909	0.1054	0.0324	0.0104	0.0106	0.0844
(3)	10 c.c.	0.5569	0.4836	0.0330	0.0101	0.0101	0.0106	0.0016

<sup>1</sup> In this series of determinations the sodium nitrite was added in solution, 1 c.c. of which contained 0.0562 gm.  $NaNO_2$ , equivalent to 0.04761 gm. NaCl (found by evaporation with several successive quantities of HCl, 0.04741 gm. NaCl).

cult the exact determination of the relatively smaller amounts of sodium contained in the urine. The first of these objections really amounts to practically nothing, inasmuch as the platinum used in such determinations can be easily recovered. Indeed, the value of the metal (platinum) is such as to warrant its recovery from the waste liquors in all such determinations, and many simple processes have been described for this purpose.<sup>8</sup> It should be borne in mind in this connection, that it is possible to separate the sodium and potassium by means of hydrochloric acid according to the method described in Treadwell,<sup>9</sup> thereby obviating the use of such large amounts of platinum chloride. The last two objections to the method, however, are deserving of more careful consideration in this connection. It therefore occurred to me to determine whether it would be possible, first, to determine the alkali metals in urine by means of sodium nitrite and hydrochloric acid by a single ignition; second, whether the sodium in urine can be determined

<sup>8</sup> See "Inorganic Preparations," RENOUP, Johns Hopkins Press, Baltimore, 1894, p. 36.

<sup>9</sup> TREADWELL: Analytical Chemistry, English translation by HALL, ii, pp. 45 and 46.

sufficiently accurately by a method involving, as does the one under consideration, the addition of relatively large amounts of a sodium salt; and third, whether nitrous acid (nitrogen trioxide gas, from arsenic trioxide and nitric acid) or aqua regia, could be employed advantageously in place of sodium nitrite and hydrochloric acid in the removal of urea from urine. As a matter of fact, it has been found possible to accurately determine the sodium, as well as the potassium in urine, after treatment with sodium nitrite and with only a single ignition; and also that the removal of urea from urine, prior to the ignition of the urine residue in the determination of the mineral constituents, can be satisfactorily accomplished by means of nitrogen trioxide gas and also by means of aqua regia. That such is the case is evident from the following determinations: 10 c.c. of solution (2) was treated with 10 c.c. of a solution of sodium nitrite, measured at 20° C. and found to weigh 10.3391 gm. and 15 c.c. of *n*/1 HCl. The solution was then evaporated to dryness on the water bath in order to get rid of the excess of acid. The residue was then dissolved in a small quantity of water, and the phosphates, together with the calcium and magnesium, removed by boiling with an excess of barium hydroxide. The excess of barium was removed from the filtrate by means of ammonia and ammonium carbonate, and the filtrate from this precipitate, together with the washings, were evaporated to small bulk in a porcelain dish, after which they were transferred to a platinum dish and evaporated to dryness and ignited over a circular burner at low red heat, keeping the dish covered with platinum foil until all of the ammonium salts had been volatilized. The residue, which was slightly grayish in color and which contained but small amounts of carbonaceous matter, was dissolved in a small amount of hot water containing a few drops of hydrochloric acid and filtered, and the filtrate and washings evaporated to dryness on the water bath in a weighed platinum dish. The residue, consisting of the chlorides of sodium and potassium, was then heated to incipient redness in the covered dish and cooled in the desiccator and weighed, after which the potassium was determined as the chlorplatinate in the usual manner. Table III gives the results of this determination.

Comparing the results of this determination with those given in Tables II and IV for solution (2), it will be seen that with the exception of the number for sodium in Table II, which is probably too low, the numbers for sodium and potassium, calculated as chlorides, agree reasonably well with one another, and differ

only within the limits of experimental error, especially when we consider the small amounts of the substances actually present in the solution. It follows, therefore, that only one ignition is required in the determination of the sodium and potassium in urine by this method, and that by exercising proper precautions with reference to the precise amount of sodium nitrite added, the quantity of sodium present in urine can be accurately determined.

TABLE III.

No. of specimen.	Quantity of urine taken.	Sodium nitrite solution added. <sup>1</sup>	NaCl + KCl found.	Weight of $K_2PtCl_6$ .	Weight of KCl found.	NaCl added in form of $NaNO_2$ .	NaCl in specimen (2).
(2)	10 c.c.	10.3391	0.6027	0.1033	0.0317	0.4729	0.0981
<sup>1</sup> 10.3261 gm. of the solution of sodium nitrite employed in this determination gave on evaporation with successive portions of hydrochloric acid, 0.4724 gm. NaCl. Hence the quantity of sodium nitrite solution used in the above determination, viz., 10.3391 gm., would give 0.4729 gm. NaCl.							

The removal of urea as an aid to the incineration of the urine residues, in the determination of the mineral constituents of urine, can also be satisfactorily accomplished by means of nitrogen trioxide gas (prepared from arsenic trioxide and nitric acid), only a single ignition being required in the determination of the alkali metals. This modification of the method under consideration has the further advantage that only small amounts of platonic chloride are required for the determination, viz., 2 to 3 cubic centimetres of a solution containing 0.1 gm. of metallic platinum per cubic centimetre, and that less washing of the residue of chlorplatينات is required to effect the separation of the sodium and potassium. That such is the case is evident from the following determinations on solutions (1) and (2), in which nitrogen trioxide gas was employed to accomplish the removal of the urea prior to ignition:

Ten cubic centimetre portions of solutions (1) and (2) were placed in Griffin beakers of 250 c.c. capacity, together with 15 c.c. of  $n/1$  HCl. The beakers were then covered with watch glasses and the contents heated nearly to boiling and a fairly rapid current of nitrogen trioxide passed into the solution as long as there was any evidence of active effervescence. (This operation should be

carried out under a hood.) Treatment of the hot solution with the gas for five to ten minutes is generally sufficient to accomplish the decomposition of the urea. The small amounts of the liquid on the under surface of the watch glasses and on the gas delivery tubes were then washed into the beakers with distilled water. The beakers were then placed on the steam bath and their contents evaporated to dryness, or nearly so, to remove any excess of acid. They were then removed from the bath and the contents dissolved in a small quantity of distilled water. The alkaline earths and phosphates were then precipitated with a slight excess of barium hydroxide, filtering and washing with hot water. The excess of barium in the filtrate was then removed with ammonia and ammonium carbonate, and the filtrate and washings from the barium carbonate precipitates evaporated to dryness in platinum dishes, after which the residues were ignited under platinum covers at low red heat until all of the ammonium salts had been volatilized. In this way grayish-white residues were obtained containing only traces or at most very small amounts of carbonaceous matter. After cooling, the grayish-white residues were treated with a few drops of dilute hydrochloric acid and a small quantity of hot water and filtered. The clear filtrates and washings were then evaporated to dryness in platinum dishes and the residues of the chlorides of the alkali metals were heated to incipient redness under platinum covers, cooled in the desiccator and weighed. The separation of the sodium and potassium was accomplished in the usual manner by means of platinum chloride. The results of these determinations on solutions (1) and (2) are given in Table IV.

It is evident from these results that treatment with nitrogen trioxide followed by the usual analytical procedure for the determination of sodium and potassium and involving only a single ignition, is sufficient to enable us to accurately determine the sodium and potassium in human urine. The only disadvantage attending the use of nitrogen trioxide in such operations as that here under consideration, is that the gas rapidly attacks the rubber connections of the apparatus employed in its preparation, thereby necessitating their frequent renewal. This of course might be overcome by constructing the apparatus required for its production entirely of glass. Up to the present, however, no attempt has been made to do this.

In this connection, however, it occurred to me that possibly aqua regia could be employed instead of nitrogen trioxide to effect the decomposition and removal of the urea as an aid to the incineration

of the urine residues at low temperatures in the determination of the metallic constituents of urine. With the object of determining whether aqua regia can decompose urea, the following experiments were carried out. An aqueous solution of urea was prepared containing approximately 2 per cent of the compound. On evaporation, 10 c.c. of this solution gave a residue weighing 0.1982 gm., and after ignition a residue weighing 0.0001 gm. 10 c.c. of the

TABLE IV.

No. of specimen.	Quantity of solution taken.	NaCl + KCl found.	K <sub>2</sub> PtCl <sub>6</sub> found.	KCl found.	KCl added to solution (2), found.	KCl actually added to solution (2).	NaCl found.
(1)	10 c.c.	0.1181	0.0748	0.0230	....	....	0.0951
(2)	10 c.c.	0.1291	0.1086	0.0333	0.0103	0.0106	0.0958

urea solution was now evaporated to dryness on the water bath and the residue treated with 20 c.c. of aqua regia (5 c.c. nitric acid and 15 c.c. hydrochloric acid), and gently heated on the water bath. A vigorous effervescence took place and after this had subsided the solution was evaporated to dryness on the water bath. The residue thus obtained was found to weigh 0.0016 gm., and after ignition, 0.0004 gm. It is evident therefore that the urea had been completely decomposed by the aqua regia.<sup>10</sup> Such being the case, the alkali metals in solutions (2) and (3) were determined in the following manner:

Ten cubic centimetres of the urine solution (2) was evaporated to dryness on the water bath. 10 c.c. of solution (3) was mixed with 10 c.c. of the 2 per cent urea solution, and the mixed solution evaporated to dryness on the water bath. To each of these residues 20 c.c. of aqua regia was added, and the resulting mixtures were then evaporated to dryness on the water bath. The residue left on the second evaporation of solution (2) was then dissolved in a small amount of water containing a few drops of hydrochloric acid, and the phosphates, together with the calcium and magnesium,

<sup>10</sup> It should be borne in mind, however, that aqua regia may dissolve small amounts of the alkali metals from glass, and hence it would seem probable that porcelain or quartz vessels could be employed to better advantage in the preliminary treatment of urine residues with aqua regia. I have not yet had the opportunity to test this point experimentally.

were removed by boiling with an excess of barium hydroxide and filtering. The excess of barium was removed by means of ammonia and ammonium carbonate, and the filtrate from the barium carbonate, together with the washings, was evaporated to dryness and gently ignited in a platinum dish. The residue was then extracted with water containing a few drops of hydrochloric acid and the solution filtered and the filtrate and washings evaporated to dryness in a platinum dish. The residue, consisting of the chlorides of the alkali metals, was then heated to incipient redness and weighed, after which the potassium was separated from the sodium by means of platinum chloride in the usual manner.

The residue left on the second evaporation of solution (3) was ignited. The residue was dissolved in water and evaporated to dryness, heated to incipient redness, and weighed. It was then dissolved in water containing a drop or two of dilute hydrochloric acid, platinum chloride added, and the potassium determined as the chloroplatinate, in the usual manner. The results of these determinations are given in Table V:

TABLE V.

No. of specimen.	Quantity of solution taken.	NaCl + KCl, found.	$K_2PtCl_6$ found.	KCl found.	KCl added to solutions, found.	KCl actually added to solutions.	NaCl found.
(2)	10 c.c.	0.1279	0.1100	0.0338	0.0108	0.0106	0.0941
(3)	10 c.c.	0.0105	0.0338	0.0104	0.0104	0.0106	....

It is evident from these results, therefore, that the alkali metals in urine can be accurately determined by preliminary treatment with aqua regia.

Finally it occurred to me that possibly the alkali metals in urine could be determined without resorting to any sort of preliminary treatment, provided that the incinerations were carried out in the usual course of removal of ammonium salts and after, rather than before, the removal of the phosphates and alkaline earths. In order to test the correctness of this supposition, the following experiments were carried out with solutions of urine (1) and (2):

Ten cubic centimetres of these solutions were precipitated by boiling with a slight excess of barium hydroxide and the excess of barium removed by means of ammonia and ammonium carbonate.

The filtrate and washings were then evaporated to small bulk in a porcelain dish and finally transferred to a platinum dish, made faintly acid with hydrochloric acid, and evaporated to dryness. The residues were then ignited at low red heat until all of the ammonium salts had been volatilized, keeping the dishes covered during the ignition. Under these conditions considerable amounts of carbonaceous matter remained in the dish, which could have been completely burned only by heating for a considerable time. The residues were treated with small amounts of water and hydrochloric acid, and filtered in order to separate the chlorides of the alkali metals from the carbonaceous matter remaining unburned. The filtrates were finally evaporated in platinum dishes and the residues heated to incipient redness, after which they were cooled and weighed. The results of these determinations are given in Table VI:

TABLE VI.

No. of specimen.	Amount of urine taken.	Weight of NaCl + KCl	Weight of $K_2PtCl_6$ .	Weight of KCl found.	Weight of KCl added, found.	Weight of KCl actually added.	Weight of NaCl found.
(1)	10 c.c.	0.1149	0.0718	0.0221	....	....	0.0928
(2)	10 c.c.	0.1288	0.1099	0.0337	0.0116	0.0106	0.0951

While the number found for the excess of potassium chloride in urine (2) is not as good as it might be, it is believed, nevertheless, that the alkali metals in urine can be determined with a reasonable degree of accuracy by the above method, involving as it does, no ignition other than that usually employed in effecting the removal of the ammonium salts. It has been found, however, that it is difficult to remove all of the carbonaceous matter, although considerably more of it is burned off at low red heat when the ignition is made after the removal of the phosphates and alkaline earths, than is ordinarily accomplished by the direct ignition of the urine residue immediately after evaporation. So far, however, as the complete incineration of the residue is concerned, this method leaves much to be desired, and on account of the difficulty of incineration, one has to choose between the danger of losing potassium by volatilization of the chloride and that of contamination with ammonium salts.

On the other hand, the first three methods above described are capable of yielding accurate results in the determination of the min-

eral constituents of urine, and each of the reagents enables one to obtain clean mineral residues from urine without the slightest difficulty. This is what the writer set out to accomplish. The choice between them is largely a matter of individual preference. Generally speaking, however, it is advantageous in all analytical operations to work with small quantities of reagents, or with reagents the excess of which can be removed by evaporation; so that on this account nitrogen trioxide or aqua regia possesses certain advantages over sodium nitrite for the preliminary treatment of urine, although the latter is a more satisfactory reagent to work with in certain other respects.

Since the above was written it has been found possible to accurately determine the alkali metals in urine by evaporating a known volume of the urine with cream of lime and igniting the residue for a short time at low red heat (not necessarily to complete incineration). The urea is thereby decomposed and removed in the form of ammonia. In the residue the alkali metals are determined by the usual method. The numbers obtained by this method for urine solutions (1) and (2) are given in Table VII.

TABLE VII.

No. of specimen.	Quantity of solution taken.	KCl + NaCl found.	$K_2PtCl_6$ found.	KCl found.	KCl added to solution (2) found.	KCl actually added to solution (2)	NaCl found.
(1)	10 c.c.	0.1153	0.0763	0.0234	....	....	0.0919
(2)	10 c.c.	0.1267	0.1091	0.0335	0.0101	0.0106	0.0932

The details of this method, which seems to be generally applicable to all animal and vegetable secretions and tissues, will be described at length in a subsequent communication.

I also propose to test the applicability of the methods above described to the determination of calcium and magnesium in urine and also to the detection and estimation of small amounts of arsenic in urine and in other animal secretions and tissues.



## HYDROLYSIS OF VETCH LEGUMIN.<sup>1</sup>

By THOMAS B. OSBORNE AND FREDERICK W. HEYL.

[From the Laboratory of the Connecticut Agricultural Experiment Station,  
New Haven, Conn.]

THE chief protein of the seeds of the vetch, *Vicia sativa*, is a globulin so closely resembling the legumin obtained from the pea, that it has not yet been possible, by a careful comparison of the properties of preparations from the two seeds, to establish any positive difference between them. We have now extended this comparison to the proportion of decomposition products which each yields on hydrolysis, and have taken much care, in conducting these analyses, to obtain results that might fairly be compared with one another. While the figures obtained show the legumins from these two seeds to be very much alike in the proportion of their decomposition products, slight differences appear between the two analyses which indicate an actual difference between the two preparations. The results of these analyses are given in the following table.

The agreement between the quantities of glycocoll, leucine, proline, phenylalanine, glutaminic acid, tyrosine, and ammonia is close, and cannot, therefore, be considered as giving any evidence of differences between the legumin from these two seeds.<sup>2</sup>

The most striking differences are shown by the figures obtained for valine, aspartic acid, and lysine. It is difficult to say how much importance should be attached to the difference found for valine since successful separations of this amino-acid are to a great extent a matter of chance. As, however, valine was very easily separated, and in relatively considerable amount, from the vetch legumin; and as it appeared to be present, if at all, in extremely small quantity,

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

<sup>2</sup> The two determinations of each of the amino acids were made by different persons so as to avoid any bias which might otherwise occur.

in the pea legumin, we are inclined to think that a real difference exists in the amount of valine yielded by these two proteins.

The difference of 2.09 per cent in the amount of aspartic acid found is considerable, and perhaps represents an actual difference between the two preparations. It must not be forgotten, however,

## HYDROLYSIS OF LEGUMIN.

	Vetch per cent.	Pea <sup>3</sup> per cent.
Glycocoll. . . . .	0.39	0.38
Alanine . . . . .	1.15	2.08
Valine . . . . .	1.36	?
Leucine . . . . .	8.80	8.00
Proline . . . . .	4.04	3.22
Phenylalanine . . . .	2.87	3.75
Aspartic acid . . . .	3.21	5.30
Glutaminic acid . . .	18.30	16.97
Serine . . . . .	?	0.53
Cystine . . . . .	not det.	not det.
Oxyproline . . . . .	" "	" "
Tyrosine . . . . .	2.42	1.55
Arginine . . . . .	11.06	11.71
Histidine . . . . .	2.94	1.69
Lysine . . . . .	3.99	4.98
Ammonia . . . . .	2.12 <sup>4</sup>	2.05 <sup>4</sup>
Tryptophane . . . .	present	present
Total . . . . .	62.65	62.22

that the ester of aspartic acid is liable to change in the presence of free caustic alkalis, and it is possible that such changes may seriously affect the results of quantitative determinations of this substance. As especial care in each case was taken, when shaking out the esters with ether, to avoid such changes, there is no reason to suppose that they occurred to any greater extent in one analysis than in the other. More extended experience is required with this determination before the comparative value of the results that are obtained can be definitely stated.

<sup>3</sup> The results of this hydrolysis have been previously published, *Journal of biological chemistry*, 1907, iii, p. 219. We have since obtained a somewhat larger amount of glutaminic acid from this protein and have also found that a much longer hydrolysis is necessary to liberate all of the basic amino acids than has heretofore been supposed. The later results for these substances are given in this table.

<sup>4</sup> OSBORNE and HARRIS: *Journal American Chemical Society*, 1903, xxv, p. 323.

The difference of one per cent in the quantity of lysine obtained is greater than is generally found between careful determinations on one and the same protein, and as other determinations of this substance, made on each of these preparations of legumin, showed a similar difference, it seems probable that pea legumin actually yields more lysine than does vetch legumin. Whether the differences shown by the figures for histidine and arginine are analytical, or depend on differences between the two proteins, cannot be definitely decided, but it is not improbable that the former is the case. The difference found for alanine is to a large extent analytical, for the vetch legumin yielded a not inconsiderable quantity of substance unquestionably consisting mostly of alanine, but which could not be converted into products of sufficient purity to weigh. No importance should be attached to the fact that serine was obtained from pea legumin, but not from vetch legumin, for it is very difficult to separate serine from most seed proteins and its isolation is largely a matter of chance.

The results of these hydrolyses make it highly probable that legumin from the pea and vetch are not identical, although they are much alike in most respects. The relations of the legumins from the horse bean and lentil to those from the pea and vetch remain to be determined; but in view of the apparent difference between the two latter it is now a serious question whether or not any two of these otherwise very similar proteins are in fact identical.

#### PREPARATION OF VETCH LEGUMIN.

Each kilogram of the finely ground seeds of the vetch was treated with three litres of 5 per cent sodium chloride solution to which was previously added 225 c.c. of cold saturated baryta, a quantity just sufficient to neutralize to litmus the acid reaction of the meal. After thorough agitation, the mass was thrown on to filters and allowed to remain until a part of the solution had been filtered through. The residues, together with the papers, were then squeezed in a powerful press and the turbid solution thus obtained, as well as the extract first filtered through the papers, was filtered perfectly clear by suction through dense filters of paper pulp. The perfectly clear extract was then dialyzed for four days until free from chlorides.

The legumin which had separated on dialysis was then filtered out, redissolved in 5 per cent sodium chloride solution and the solu-

tion which resulted filtered through paper pulp and again dialyzed for four days. The precipitate produced by dialysis was filtered out and washed carefully by suspending in distilled water, all lumps being broken up by passing the suspension through fine bolting cloth. After sucking the legumin as dry as possible on a paper on a Buchner funnel, it was dehydrated by treating in the same way with absolute alcohol, and after digesting with dry ether, was freed from ether by placing it in a desiccator over sulphuric acid.

As legumin is the only protein that is precipitated by dialysis, which can be obtained from the vetch, the above process is sufficient to separate it completely from the legumelin and proteose which are also present in this seed.

Thus prepared, vetch legumin forms a fine, dusty powder with the properties and composition described in previous papers from this laboratory.<sup>5</sup>

#### HYDROLYSIS OF VETCH LEGUMIN.

Six hundred grams of the legumin, equivalent to 520 gm. ash- and moisture-free protein, were digested on the water bath with a mixture of 600 c.c. water and 600 c.c. of concentrated hydrochloric acid, and boiled in an oil bath for twenty-seven hours. The hydrolysis solution was then concentrated to a thick syrup under diminished pressure, and esterified according to the directions of Emil Fischer. After shaking out the esters with ether in the usual manner, the aqueous layer was freed from inorganic salts, and a second esterification was carried out. After removing the ether on the water bath at 760 mm., the esters were fractionated as follows:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	55°	15.00 mm.	15.26 gm.
II	100°	10.00 "	30.60 "
III	110°	0.67 "	107.98 "
IV	150°	1.78 "	56.56 "
V	200°	2.00 "	74.66 "
Total . . . . .			285.06 gm.

The undistilled residue weighed 63 gm.

**Fraction I.** — This fraction, together with the ether distilled from the esters, yielded 3.77 gm. glycocoll ethyl ester hydrochloride,

<sup>5</sup> OSBORNE and CAMPBELL: *Journal of the American Chemical Society*, 1896, xviii, p. 583; *Ibid.*, 1898, xx, p. 406; *Ibid.*, 1898, xx, p. 410.

which when recrystallized from alcohol formed the characteristic needles which melted at  $145^{\circ}$  to a clear oil.

*Chlorine*, 0.1388 gm. subst., gave 0.1429 gm. AgCl.

Calculated for  $C_4H_{10}O_2NCl = Cl$  25.45 per cent.

Found . . . . . = Cl 25.45 " "

The amino acids were regenerated in the filtrate from the glyco-coll ester hydrochloride and added to Fraction II.

**Fraction II.**— This fraction was saponified by boiling with water for ten hours, the solution evaporated to dryness under diminished pressure, and proline removed by extracting with alcohol in the usual manner. The amino acids insoluble in alcohol, together with those from Fraction I, yielded, after a systematic fractional crystallization, 6.24 gm. of leucine, 4.89 gm. of valine, and 5.99 gm. of alanine. Glycocoll was not present in this fraction. The leucine was analyzed as follows:

*Carbon and hydrogen*, 0.3034 gm. subst., gave 0.6121 gm.  $CO_2$  and 0.2754 gm.  $H_2O$ .

Calculated for  $C_6H_{11}O_2N = C$  54.96; H 9.92 per cent.

Found . . . . . = C 55.01; H 10.08 " "

The valine crystallized in plates having the characteristic mother-of-pearl lustre, and appeared homogeneous under the microscope.

*Carbon and hydrogen*, 0.1757 gm. subst., gave 0.3300 gm.  $CO_2$  and 0.1463 gm.  $H_2O$ .

Calculated for  $C_6H_{11}O_2N = C$  51.28; H 9.40 per cent.

Found . . . . . = C 51.22; H 9.25 " "

The immediately following fraction obtained from the filtrate of the material giving the above analytical data was analyzed with the following result:

*Carbon and hydrogen*, 0.1223 gm. subst., gave 0.2294 gm.  $CO_2$  and 0.1035 gm.  $H_2O$ .

Calculated for  $C_6H_{11}O_2N = C$  51.28; H 9.40 per cent.

Found . . . . . = C 51.15; H 9.40 " "

The alanine crystallized in characteristic needles which decomposed at  $290^{\circ}$ . When crystallized from water it formed dense, hard prisms, giving the following analysis:

*Carbon and hydrogen*, 0.1974 gm. subst., gave 0.2933 gm. CO<sub>2</sub> and 0.1452 gm. H<sub>2</sub>O.

Calculated for C<sub>8</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.45; H 7.86 per cent.

Found . . . . . = C 40.52; H 8.12 " "

**Fraction III**— This fraction was saponified in the usual way and evaporated to dryness under reduced pressure. Proline was extracted with boiling absolute alcohol. The amino acids, insoluble in alcohol, consisted chiefly of leucine which weighed 38.42 gm.

*Carbon and hydrogen*, 0.3178 gm. subst., gave 0.6363 gm. CO<sub>2</sub> and 0.2825 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>12</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . . = C 54.60; H 9.85 " "

The proline extracts contained 20.98 gm. proline which was weighed in the form of the copper salts. The racemic-proline copper crystallized from water in characteristic plates.

*Water*, 0.2545 gm. lost 0.0275 gm. H<sub>2</sub>O at 110°.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu = H<sub>2</sub>O 10.99 per cent.

Found . . . . . = H<sub>2</sub>O 10.80 " "

*Copper*, 0.2270 gm. subst. (dried at 110°), gave 0.0626 gm. CuO.

Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>Cu = Cu 21.81 per cent

Found . . . . . = Cu 22.03 " "

The lævo-proline copper was freed from copper and converted into the characteristic phenylhydantoin, which crystallized in prisms melting sharply at 142°–143°.

*Carbon and hydrogen*, 0.1279 gm. subst., gave 0.3133 gm. CO<sub>2</sub> and 0.0646 gm. H<sub>2</sub>O.

*Nitrogen*, 0.2180 gm. subst., required 19.7 N/10 HCl.

Calculated for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub> = C 66.67; H 5.57; N 12.96 per cent.

Found . . . . . = C 66.80; H 5.61; N 12.65 " "

After separating the above quantity of leucine, the residue of amino acids yielded 1.6 gm. of aspartic acid in characteristic crystals which, at 300°, reddened but did not melt. The remaining acids, from which nothing definite could be separated, were then converted into copper salts and 2.37 gm. of copper aspartate were obtained.

*Copper*, 0.1202 gm. subst., gave 0.0352 gm. CuO.

*Nitrogen*, 0.5000 gm. subst., required 19.3 c.c. N/10 HCl.

Calculated for  $C_4H_5O_4NCu \cdot \frac{1}{2} H_2O = Cu$  23.07; N 5.08 per cent.

Found . . . . . = Cu 23.39; N 5.40 " "

The rest of the copper salts were dried and extracted with boiling absolute alcohol in which much of them dissolved. After removing the copper from these two parts and fractioning the free acids separately, there were obtained 1.09 gm. leucine which contained 54.86 per cent carbon and 2.21 gm. of valine.

*Carbon and hydrogen*, 0.1487 gm. subst., gave 0.2805 gm.  $CO_2$  and 0.1228 gm.  $H_2O$ .

Calculated for  $C_5H_{11}O_2N = C$  51.28; H 9.40 per cent.

Found . . . . . = C 51.44; H 9.18 " "

All the different fractions of valine which had been weighed were united and 0.3908 gm. were dissolved in 20 per cent HCl, the final volume being 17.94 c.c. The solution gave a rotation of  $+1.2^\circ$  in a two decimetre tube at  $20^\circ$ , from which is calculated:

$$(a) \frac{20^\circ}{D} = +28.3^\circ.$$

E. Schulze<sup>6</sup> found for valine from lupine germlings  $+28.2^\circ$  and  $+27.9^\circ$ , and E. Fischer<sup>7</sup> found  $+27.1^\circ$  for a preparation from casein. The valine was racemized in the usual manner by heating with baryta in the autoclave for twenty-four hours at  $175^\circ$ . It was then coupled with phenylisocyanate in alkaline solution and the hydantoic acid recrystallized from water. The substance separated in the characteristic hexagonal plates which melted at  $161^\circ$ .

*Carbon and hydrogen*, 0.1540 gm. subst., gave 0.3427 gm.  $CO_2$  and 0.1032 gm.  $H_2O$ .

Calculated for  $C_{12}H_{16}O_5N_2 = C$  60.95; H 6.84 per cent.

Found . . . . . = C 60.68; H 7.44 " "

Alanine appeared to be present in considerable amount, but no product of sufficient purity to weigh could be obtained.

**Fraction IV.**—This fraction yielded by the usual methods 8.73 gm. phenylalanine hydrochloride. The free phenylalanine decomposed at about  $270^\circ$ .

<sup>6</sup> SCHULZE, E.: *Zeitschrift für physiologische Chemie*, 1902, xxxv, p. 301.

<sup>7</sup> FISCHER, E.: *Ibid.*, 1902, xxxvii, p. 159.

*Carbon and hydrogen*, 0.1909 gm. subst., gave 0.4572 gm. CO<sub>2</sub> and 0.1168 gm. H<sub>2</sub>O.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>4</sub>N = C 65.45; H 6.66 per cent.

Found . . . . . = C 65.32; H 6.79 " "

There was further obtained 6.63 gm. aspartic acid as the barium salt, 9.18 gm. glutaminic acid hydrochloride, and 11.10 gm. copper aspartate (air dried). The aspartic acid reddened but did not decompose at 300°.

*Carbon and hydrogen*, 0.2566 gm. subst., gave 0.3370 gm. CO<sub>2</sub> and 0.1248 gm. H<sub>2</sub>O.

Calculated for C<sub>4</sub>H<sub>7</sub>O<sub>4</sub>N = C 36.09; H 5.26 per cent.

Found . . . . . = C 35.81; H 5.40 " "

The copper aspartate crystallized from a large volume of water in tyrosine-like sheaves.

*Nitrogen*, 0.1780 gm. subst., required 6.6 cc. N/10 HCl.

*Copper*, 0.1226 gm. subst., gave 0.0353 gm. CuO.

Calculated for C<sub>4</sub>H<sub>7</sub>O<sub>4</sub>NCu 41/2 H<sub>2</sub>O = Cu 23.07; N 5.08 per cent.

Found . . . . . = Cu 23.00; N 5.19 " "

**Fraction V.**— This fraction yielded 9.52 gm. of phenylalanine hydrochloride, 33.12 gm. glutaminic acid as barium salt and as hydrochloride, and 3.97 gm. copper aspartate. The glutaminic acid melted at 202°–203°.

*Carbon and hydrogen*, 0.2365 gm. subst., gave 0.3535 gm. CO<sub>2</sub> and 0.1323 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . . = C 40.76; H 6.21 " "

The copper aspartate was analyzed as follows:

*Copper*, 0.1273 gm. subst., gave 0.0369 gm. CuO.

Calculated for C<sub>4</sub>H<sub>7</sub>O<sub>4</sub>NCu 41/2 H<sub>2</sub>O = Cu 23.07 per cent.

Found . . . . . = Cu 23.16 " "

#### GLUTAMINIC ACID.

Glutaminic acid appears to separate from the products of hydrolysis of legumin with great uncertainty, and it seems to be a matter of chance to secure conditions favorable for its isolation.

Thus all attempts to separate the glutaminic acid directly from



the products of the hydrolysis just described were complete failures, whereas from a similar hydrolysis of 867.2 gm. ash and moisture-free legumin, we obtained 189.1 gm. of the hydrochloride and from the remaining solution of esterification 9.10 gm. more, making 198.2 gm. equal to 18.30 per cent of the free acid. This result is higher than the 16.48 per cent found by Osborne and Gilbert,<sup>8</sup> but must be accepted as the more nearly correct, for the hydrochloride weighed in this last determination was very pure. The free glutaminic acid obtained from this hydrochloride gave the following analysis:

*Carbon and hydrogen*, 0.2049 gm. subst., gave 0.3070 gm. CO<sub>2</sub> and 0.1162 gm. H<sub>2</sub>O.

Calculated for C<sub>8</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . . = C 40.86; H 6.10 " "

#### TYROSINE.

A quantity of legumin equal to 43.35 gm. ash and moisture free, was hydrolyzed for twenty-three hours by boiling in an oil bath with three times its weight of sulphuric acid and six times its weight of water. The solution freed from sulphuric acid and concentrated to a moderate volume, yielded a crystalline separation which when recrystallized gave 1.0490 gm. of tyrosine = 2.42 per cent.

*Nitrogen*, 0.4895 gm. subst., required 3.72 c.c. 5/7 N-HCl.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>3</sub>N = N 7.73 per cent.

Found . . . . . = N 7.60 " "

The filtrate from the tyrosine was used for determinations of the bases according to the method of Kossel and Patten.

#### HISTIDINE.

The solution of the histidine = 500 c.c.

*Nitrogen*, 50 c.c. required 3.45 c.c. 5/7 N-HCl = 0.0345 gm. N = 0.3450 gm. in 500 c.c. = 1.2730 gm. histidine = 2.94 per cent.

The histidine was converted into the dichloride for identification. It decomposed at 233° and gave the following analysis:

<sup>8</sup> OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

*Nitrogen*, 0.2250 gm. subst., gave 34.21 c.c. N at 11° and 753.4 mm.

Calculated for  $C_6H_9O_2N_2HCl = N$  18.47 per cent.

Found . . . . . = N 18.20 " "

*Chlorine*, 0.1139 gm. subst., gave 0.1436 gm. AgCl.

Calculated for  $C_6H_9O_2N_2HCl = Cl$  31.14 per cent.

Found . . . . . = Cl 31.18 " "

#### ARGININE.

The solution of the arginine = 1000 c.c.

*Nitrogen*, 50 c.c. required 7.54 c.c. 5/7 N-HCl = 0.0754 gm. N = 1.5080 gm.

N in 1000 c.c. = 4.6854 gm. arginine + 0.1080 gm. = 4.7934 gm. =  
11.06 per cent.

The arginine was converted into the nitrate for identification.

*Nitrogen*, 0.2490 gm. subst., gave 60.25 c.c. N at 12° and 752.1 mm.

Calculated for  $C_6H_{14}O_4N_4HNO_3 \cdot 1/2 H_2O = N$  28.49 per cent.

Found . . . . . = N 28.81 " "

#### LYSINE.

The lysine picrate weighed 4.1277 gm. = 1.6069 gm. lysine  
= 3.70 per cent. The lysine was identified as the picrate.

*Nitrogen*, 0.4301 gm. subst., required 7.95 c.c. 5/7 N-HCl.

Calculated for  $C_6H_{14}O_2N_2 - C_6H_5O_7N_3 = N$  18.70 per cent.

Found . . . . . = N 18.50 " "

The results of two other determinations of lysine agreed closely,  
namely, 3.80 and 3.99 per cent.

#### CYSTINE.

Owing to the small proportion of sulphur in legumin, no attempt  
was made to isolate cystine.

## HYDROLYSIS OF CHICKEN MEAT.<sup>1</sup>

BY THOMAS B. OSBORNE AND FREDERICK W. HEYL.

[From the Laboratory of the Connecticut Agricultural Experiment Station.]

IT is a matter of interest to know whether the meats which are commonly used as foods differ in any marked degree from the proteins of seeds as well as from one another, in respect to the proportion of the amino-acids yielded by them when hydrolyzed. The only data relating to this question that we have found in the literature are furnished by one hydrolysis, by Abderhalden and Sasaki,<sup>2</sup> of syntonin from ox muscle, and by determinations of the bases in this substance by Hart.<sup>3</sup> We have accordingly undertaken to determine quantitatively the products of hydrolysis of the muscle substance of several other species of animals, as we thought it not improbable that differences exist between the muscle substances of different species of animals similar to those found between the proteins of seeds of different species of plants.

For this work we have thought best to employ the entire muscle substance, with the removal only of water-, alcohol-, and ether-soluble substances. The material for our hydrolysis of chicken muscle was prepared by removing the edible parts of mature hens as completely as possible, *immediately* after they had been killed and bled. After carefully separating adherent fat and connective tissue, the remaining muscle was chopped fine and suspended over night in a liberal quantity of water. The solid substance was then strained out and pressed, and the treatment with water repeated. The residue was then digested once with 95 per cent alcohol, and twice with absolute alcohol. After removing the alcohol by strongly pressing, the residue was digested with a large amount of ether until most

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

<sup>2</sup> ABDERHALDEN and SASAKI: *Zeitschrift für physiologische Chemie*, 1907, li, p. 409.

<sup>3</sup> HART: *Ibid.*, 1901, xxxiii, p. 348.

of the fat was extracted, and the ether was then removed by filtration and exposure to dry air.

The results of the hydrolysis of this material are given in the following table, and for comparison those published by Abderhalden and Sasaki and by Hart for syntonin from ox muscle.

	Chicken muscle per cent.	Ox syntonin per cent.
Glycocoll . . . . .	0.68	0.50 <sup>4</sup>
Alanine . . . . .	2.28	4.00 <sup>4</sup>
Valine . . . . .	?	0.90 <sup>4</sup>
Leucine . . . . .	11.19	7.80 <sup>4</sup>
Proline . . . . .	4.74	3.30 <sup>4</sup>
Phenylalanine . . . . .	3.53	2.50 <sup>4</sup>
Aspartic acid . . . . .	3.21	0.50 <sup>4</sup>
Glutaminic acid . . . . .	16.48	13.60 <sup>4</sup>
Serine . . . . .	?	?
Cystine . . . . .	not determined	not determined
Oxyproline . . . . .	not determined	not determined
Tyrosine . . . . .	2.16	2.20
Arginine . . . . .	6.50	5.06 <sup>5</sup>
Histidine . . . . .	2.47	2.66 <sup>5</sup>
Lysine . . . . .	7.24	3.26 <sup>5</sup>
Ammonia . . . . .	1.67	0.83 <sup>5</sup>
Tryptophane . . . . .	present	not determined
Total . . . . .	62.15	47.11

The differences in the proportion of several of the amino-acids obtained from the muscle substance of these two species of animals are considerable, and indicate a marked difference between them. It is to be noted, however, that a very considerable difference is shown by the summation of the two analyses, and it is therefore doubtful whether or not the two analyses can be fairly compared. The differences in most cases considerably exceed those that are usually due to imperfections in the methods of separation and isolation. Whether or not differences in the nature of the material employed for the two analyses are responsible for the differences in the results obtained cannot be determined without further study, and definite conclusions as to differences in the muscle substance of these two species must await the determinations which we have in view, made on material prepared under uniform conditions.

<sup>4</sup> ABDERHALDEN and SASAKI: *Loc. cit.*

<sup>5</sup> HART: *Loc. cit.*

The most striking feature of our hydrolysis of chicken muscle is the relatively large amount of lysine found. This determination is certainly not too high, for the lysine picrate which we weighed was exceedingly pure. Except for this higher yield of lysine these results are very similar to those obtained with most of the proteins from leguminous seeds.

## HYDROLYSIS OF CHICKEN MUSCLE.

There were taken for this hydrolysis 274.8 gm. of ash- and moisture-free meat. This was suspended in a mixture of 600 c.c. of water and 600 c.c. of hydrochloric acid, sp. gr. 1.19. Solution was effected by heating on the boiling water bath for five hours, and the hydrolysis was completed by boiling in an oil bath for seventeen hours. The hydrolysis solution was concentrated and saturated with hydrochloric acid gas at 0°, and after standing five days on ice, there was obtained in the usual manner, 47.91 gm. of glutaminic acid hydrochloride, which, after deducting the ammonium chloride which this contained, was equivalent to 38.39 gm. of the free acid or 13.96 per cent of the protein. The free glutaminic acid decomposed at about 203° with effervescence.

*Carbon and hydrogen*, 0.2666 gm. subst., gave 0.4006 gm. CO<sub>2</sub> and 0.1445 gm. H<sub>2</sub>O.

Calculated for C<sub>8</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . = C 40.98; H 6.02 " "

The filtrate from the above direct determination of glutaminic acid hydrochloride was concentrated sharply to a heavy syrup and esterification was carried out as usual. Three esterifications were made, but the third yielded but a few gm. of ether-soluble material. The ether solution of the amino-esters was dried for about three weeks over anhydrous sodium sulphate, and the ether was removed on the boiling water bath at ordinary pressures. The residual esters were fractionally distilled with the following results:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	95°	10.00 mm.	25.50 gm.
II	104°	0.48 "	60.95 "
III	136°	0.41 "	35.88 "
IV	200°	0.21 "	29.03 "
Total . . . . .			151.36 gm.

The undistilled residue weighed 27 gm.

**Fraction I.**— This fraction was saponified by boiling with water for seven hours, and the solution was evaporated to dryness under diminished pressure. The amino-acids weighed about 19.1 gm. Proline was extracted with boiling absolute alcohol. The amino-acids insoluble in alcohol weighed 17.9 gm. There were obtained 3.50 gm. glycocoll ester hydrochloride which melted at 145°.

*Nitrogen*, 0.1549 gm. subst., required 11.1 c.c. N/10 HCl.

*Chlorine*, 0.1598 gm. subst., gave 0.1619 gm. AgCl.

Calculated for  $C_4H_{10}O_2NCl = Cl\ 25.45$ ;  $N\ 10.04$  per cent.

Found . . . . . =  $Cl\ 25.05$ ;  $N\ 10.03$  " "

The more insoluble part of the fraction yielded 4.92 gm. leucine.

*Carbon and hydrogen*, 0.1494 gm. subst., gave 0.3013 gm.  $CO_2$  and 0.1301 gm.  $H_2O$ .

Calculated for  $C_6H_{12}O_2N = C\ 54.96$ ;  $H\ 9.92$  per cent.

Found . . . . . =  $C\ 55.00$ ;  $H\ 9.67$  " "

The alanine weighed 6.28 gm. It crystallized from water in prisms and from dilute alcohol in the characteristic bundles of needles. It decomposed at about 290°.

*Carbon and hydrogen*, 0.2130 gm. subst., gave 0.3193 gm.  $CO_2$  and 0.1504 gm.  $H_2O$ .

Calculated for  $C_3H_7O_2N = C\ 40.45$ ;  $H\ 7.86$  per cent.

Found . . . . . =  $C\ 40.88$ ;  $H\ 7.84$  " "

Valine seemed to be present but could not be isolated in a condition suitable for identification.

**Fraction II.**— This fraction was saponified and the proline was removed as usual. The alcoholic solutions of the proline from fractions I and II were joined and evaporated to dryness under reduced pressure. The residue was boiled with absolute alcohol and allowed to stand over night, when 1.40 gm. of substance separated. This was filtered off and the filtrate when again subjected to the same process was wholly soluble in absolute alcohol. The proline, weighed as the copper salt, was equal to 13.04 gm. For identification the phenylhydantoine of the lævo-proline was used. It crystallized from much water in large prisms, melting at 143° sharply.

*Carbon and hydrogen*, 0.1530 gm. subst., gave 0.3730 gm.  $CO_2$  and 0.0797 gm.  $H_2O$ .

*Nitrogen*, 0.2212 gm. subst., required 20.4 c.c. N/10 HCl.

Calculated for  $C_{12}H_{12}O_2N_2 = C\ 66.67$ ;  $H\ 5.57$ ;  $N\ 12.96$  per cent.

Found . . . . . =  $C\ 66.48$ ;  $H\ 5.78$ ;  $N\ 12.91$  " "

The amino-acids insoluble in alcohol weighed 32.12 gm., and yielded 25.83 gm. of leucine.

*Carbon and hydrogen*, 0.1445 gm. subst., gave 0.2909 gm.  $\text{CO}_2$  and 0.1314 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_9\text{H}_{18}\text{O}_2\text{N} = \text{C } 54.96$ ;  $\text{H } 9.92$  per cent.

Found . . . . =  $\text{C } 54.90$ ;  $\text{H } 10.10$  " "

**Fraction III.**— From this fraction phenylalanine was extracted in the usual manner with ether, and the aqueous layer added to the corresponding aqueous layer of Fraction IV. There were obtained 3.78 gm. of phenylalanine hydrochloride.

*Carbon and hydrogen*, 0.1382 gm. subst., gave 0.3338 gm.  $\text{CO}_2$  and 0.0835 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_9\text{H}_{11}\text{O}_2\text{N} = \text{C } 65.45$ ;  $\text{H } 6.66$  per cent.

Found . . . . =  $\text{C } 65.87$ ;  $\text{H } 6.71$  " "

**Fraction IV.**— This fraction yielded 8.08 gm. of phenylalanine hydrochloride, 5.52 gm. of aspartic acid, 8.61 gm. of glutaminic acid hydrochloride and 6.84 gm. of copper aspartate. The aspartic acid reddened but did not decompose at  $300^\circ$ .

*Carbon and hydrogen*, 0.2498 gm. subst., gave 0.3296 gm.  $\text{CO}_2$  and 0.1256 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_4\text{H}_7\text{O}_4\text{N} = \text{C } 36.09$ ;  $\text{H } 5.26$  per cent.

Found . . . . =  $\text{C } 35.98$ ;  $\text{H } 5.58$  " "

The copper aspartate was analyzed as follows:

*Copper*, 0.1205 gm. subst., gave 0.0343 gm.  $\text{CuO}$ .

*Nitrogen*, 0.4692 gm. subst., required 17.8 c.c.  $\text{N}/10 \text{ HCl}$ .

Calculated for  $\text{C}_4\text{H}_5\text{O}_4\text{NCu } 4\frac{1}{2} \text{ H}_2\text{O} = \text{Cu } 23.07$ ;  $\text{N } 5.08$  per cent.

Found . . . . . =  $\text{Cu } 22.74$ ;  $\text{N } 5.31$  " "

The glutaminic acid hydrochloride when decomposed with an equivalent quantity of potassium hydroxide yielded free glutaminic acid which decomposed at  $202^\circ$ . The undistilled residue was worked up for glutaminic acid hydrochloride in the usual manner, but none separated, even on prolonged standing.

#### TYROSINE.

A quantity of substance equal to 36.64 gm., ash- and moisture-free, was hydrolyzed by boiling for eighteen and one half hours in

a mixture of 81 c.c. sulphuric acid and 300 c.c. water. The solution was freed from sulphuric acid with baryta, and the filtrate and washings from the barium sulphate were concentrated, yielding 0.791 gm. pure tyrosine, equal to 2.16 per cent.

*Carbon and hydrogen*, 0.1667 gm. subst., gave 0.3635 gm.  $\text{CO}_2$  and 0.0945 gm.  $\text{H}_2\text{O}$ .

*Nitrogen*, 0.1642 gm. subst., required 9.25 c.c. N/10 HCl.

Calculated for  $\text{C}_9\text{H}_{11}\text{O}_3\text{N}$  = C 59.67 ; H 6.08 ; N 7.73 per cent.

Found . . . . = C 59.46 ; H 6.26 ; N 7.88 " "

The filtrate and washings from the tyrosine were worked up for bases according to the method of Kossel and Patten, with the following results: ..

#### HISTIDINE.

The solution of the histidine = 500 c.c.

*Nitrogen*, 50 c.c. solution required 2.45 c.c. 5/7 N-HCl = 0.2450 gm. N in 500 c.c. = 0.9045 gm. histidine = 2.47 per cent.

The histidine was converted into the dichloride. It crystallized in prisms, melting very sharply at  $233^\circ$ .

*Chlorine*, 0.0720 gm. subst., gave 0.0898 gm. AgCl.

Calculated for  $\text{C}_8\text{H}_{11}\text{O}_2\text{N}_2\text{Cl}_2$  = Cl 31.14 per cent.

Found . . . . = Cl 30.84 " "

#### ARGININE.

The solution of the arginine = 1000 c.c.

*Nitrogen*, 50 c.c. solution required 3.60 c.c. 5/7 N-HCl = 0.7200 gm. N in 1000 c.c. = 2.2370 gm. arginine + 0.1440 gm. = 2.3810 gm. = 6.50 per cent.

The arginine was converted into the copper nitrate double salt.

*Copper*, 0.1065 gm. subst. (air dried), gave 0.0144 gm.  $\text{CuO}$ .

Calculated for  $\text{C}_{12}\text{H}_{28}\text{O}_4\text{N}_4\text{Cu}(\text{NO}_3)_2 \cdot 3 \text{H}_2\text{O}$  = Cu 10.79 per cent.

Found . . . . = Cu 10.80 " "

#### LYSINE.

The lysine picrate weighed 6.2810 gm. = 2.6554 gm. lysine = 7.24 per cent.



Nitrogen, 0.1682 gm. subst., required 22.3 c.c. N/10 HCl.

Calculated for  $C_6H_{14}O_2N_2C_6H_5O_7N_2 = N$  18.67 per cent.

Found . . . . . = N 18.56 " "

#### DISTRIBUTION OF NITROGEN.

One gm. of the chicken meat, equal to 0.9158 gm. ash- and moisture-free, was hydrolyzed, and the ammonia and nitrogen precipitable by phosphotungstic acid were determined according to Hausmann's method as modified by Osborne and Harris.<sup>6</sup>

The results obtained were:

	Per cent.
N as ammonia . . . . .	1.20
N precipitated by phosphotungstic acid. . . . .	4.82
N in magnesia precipitate . . . . .	0.44
Other N by difference . . . . .	9.63
Total N . . . . .	16.09

The nitrogen as ammonia is equal to 1.67 per cent of ammonia. The nitrogen contained in the quantities of histidine, arginine, and lysine found by hydrolyzing the chicken meat is equal to 4.15 per cent, which is 0.67 per cent less than that precipitated by phosphotungstic acid. This difference is probably largely caused by non-protein basic substances contained in the meat.

<sup>6</sup> OSBORNE and HARRIS : Journal of the American Chemical Society, 1903, xxv, p. 323.

## CONTROL OF SPASMS BY ASPHYXIATION.

BY A. H. RYAN AND C. C. GUTHRIE.

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Saint Louis, Missouri.]

### INTRODUCTION.

MANY procedures have been suggested and tried in the control of spasms ordinarily considered as being due to a toxin acting upon the central nervous system, as in tetanus, strychnine poisoning, etc. So far no such method has given permanent results.

In spasms the motor cells are considered to be involved and are therefore more fatigued than ordinarily. Assuming that in partial asphyxiation the cells most fatigued would succumb first, we were led to believe that this method might offer a means for temporarily controlling spasms. The following experiments were performed to determine in how far this was possible.

### EXPERIMENTS.

Frogs weighing 21 gm. were used. Each was given one drop of 1.0 per cent strychnine by the mouth and then placed in a bottle under the conditions shown in the following table. The results are also given in the table.

Similar results were obtained on a cat as shown in the following protocol (Protocol 1).

#### PROTOCOL 1.

Adult gray cat.

- |       |       |   |
|-------|-------|---|
| 4.13  | P. M. | Administered 1.0 c.c. 0.1 (?) per cent strychnine by mouth.           |
| 4.22  | " "   | Strong spasms.  |
| 4.23  | " "   | Placed in atmosphere of CO <sub>2</sub> . Relaxed almost immediately. |
| 4.24½ | " "   | Returned to air. Rapid recurrence of spasms.                          |
| 4.26  | " "   | Placed in CO <sub>2</sub> .   |
| 4.29  | " "   | Relaxed.  |
| 4.33  | " "   | Respiration stopped. Placed in air. Gave artificial respiration.      |
| 4.35½ | " "   | Respiration restored.   |

TABLE I.

	1. Frog in atmosphere of air.	2. Frog in atmosphere of air, but bottle corked.	3. Frog in atmosphere of CO <sub>2</sub> .	4. Frog in atmos. of Hydrogen.	5. Frog in atmos- phere of Oxygen.
Time Strychnine given. Interval before first spasm. Condition at 2:59 P.M.	2:45'30" P.M. 7½ min. ....	2:46' P.M. 11 min. ....	2:46' 15" P.M. 12½ min. ....	2:46' 45" P.M. 7½ min. Placed in CO <sub>2</sub> to control spasms.	2:47' 15" P.M. 5½ min.
" " 3:11 P.M.	Stiff. Thrown into spasms by handling. Give spasms on touching and remain in tetanus. No change. Strong res- piratory movements.	Handled without spasms. Resp. movement stopped. Slight spasms on shaking, but relax quickly. No change.	Same as 2.	Same as 2.	Same as 1.
" " 3:18 P.M.			No spasms on shaking vigorously.	Same as 2.	Same as 1.
" " 3:21 P.M.			Placed in air. No res- piratory movements.	Same as 3.	Same as 1.
" " 3:25 P.M.	In tetanus.	....	Spasms and respiratory mov'ts replaced in CO <sub>2</sub> . Respiratory movements stopped still in tetanus.	....	Same as 1.
" " 3:28 P.M.	....	Relaxed but slight spasms can be elicited.	....	Breathing occasionally.	
" " 3:30 P.M.	....	....	Relaxing.	Spasms begin- ning. Breathing	
" " 3:32 P.M.	....	....	....		
" " 3:34 P.M.	....	....	....		
" " 3:35 P.M.	....	No spasms except on strong stimulation.	Same as 2.	Spasms on slight stimulation. Placed again in CO <sub>2</sub> .	Same as 1.
" " 3:41 P.M.	....	....	....		
" " 3:53 P.M.	No change	No change.	Completely relaxed		
" " 4:16 P.M.	....	....	Placed in air.	Completely relaxed.	
" " 4:23 P.M.	....	....	Strong spasms. Breathing.		Oxygen Renewed.
" " 4:43 P.M.	....	....	Placed in CO <sub>2</sub> .	....	
" " 4:52 P.M.	....	....	Completely relaxed.	Completely relaxed	
" " 6:00 P.M.	In tetanus.	Relaxed almost completely, but excitable.	Completely relaxed.		Dead.

- 4.36 P. M. Spasms.
- 4.36½ " " Placed in CO<sub>2</sub>.
- 4.38 " " Relaxed.
- 4.40 " " Pulse and respiratory movements stopped.

Through the courtesy of Dr. C. B. Davenport of the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y., we had the opportunity of testing the efficiency of a magnesium sulphate solution on a tetanic sheep. The results are given in the following protocol (Protocol 2).

#### PROTOCOL 2.

Cold Spring Harbor, N. Y., April 9, 1908. Adult sheep found in pen in morning with all symptoms of tetanus. One hundred and fifty to one hundred and sixty pounds weight (estimated). About ten days previously gave birth to a large dead fetus.

- 9.07 A. M. Began subcutaneous injection of 400 c.c. 25 per cent MgSO<sub>4</sub>.
- 9.15 " " Finished injection.
- 9.25 " " Slight relaxation of muscles of jaws. Spasms continue at intervals at least as strong as ever.
- 9.30 " " Breathing slower and easier. Rate 93 per minute.
- 9.38 " " Rate 48 per minute. Muscles well relaxed. Slight spasms. Tongue blue. Eye reflex strong (touch). No, or slight, dilation of pupil.
- 9.43 " " Respiration ceased. Heart still beating. Several slight and ineffectual respiratory movements seen.

#### DISCUSSION.

In every case of spasms by strychnine poisoning in frogs, relaxation promptly followed the placing of the animal in an atmosphere of carbon dioxide, while the reverse was true when the animal was taken from carbon dioxide and placed in an atmosphere of air. The frog in an atmosphere of oxygen was first to show the effects of the strychnine. A second series of frogs gave practically the same results.

In the case of mammals the periods of relaxation were necessarily much shorter because of the greater susceptibility of mammals to asphyxia. By mixing carbon dioxide and air in proper proportions it is possible that mammals might be kept in a relaxed condition for a longer period.

In the sheep experiment the spasms were controlled in that the animal relaxed and became perfectly limp; but from the enormous dose, blue tongue and failure of respiration, it seems probable that the result was not due to a specific action of the magnesium salt. The dose in this case was about 1.25 gm. per kilo of body weight. The dose found to be most efficient by Meltzer and Auer<sup>1</sup> in producing anesthesia is about 1.50 gm. per kilo of body weight. The size of the dose is not so striking when administered to small animals such as rabbits and cats, but when figured for a 70 kilo animal it takes on enormous proportions, in fact being larger than the therapeutic dose of the same drug by the mouth. On the basis of 1.50 gm. per kilo of body weight the concentration of the magnesium salt in the blood would be about 5 per cent. But assuming that only half of it were absorbed it is conceivable that even then the character of the blood would be altered to such an extent that the normal processes of respiration would be interfered with. The nervous system would be first to succumb and especially those elements which were in the greatest state of fatigue, so that relaxation and anesthesia would follow as a secondary effect rather than as the result of a specific action of the salt solution.

It is interesting to consider the possible relationship of these results and those of the numerous investigators who have endeavored to control spasms by the injection of various salt solutions. S. A. Matthews<sup>2</sup> found that the convulsions produced in tetanus could be inhibited by the intravenous injection of a salt solution in which calcium was present. MacCallum and Voegtlin<sup>3</sup> similarly inhibited tetany caused by removal of the parathyroids by injection of a solution of calcium chloride. Along a similar line is the work of Meltzer and Auer<sup>1</sup> on the anesthetic properties of magnesium salts.

#### CONCLUSIONS.

It is evident from the above results that spasms can at least be temporarily controlled in amphibians and mammals by partial asphyxiation.

<sup>1</sup> MELTZER and AUER : This journal, 1905, xiv, p. 366; 1906, xv, p. 387.

<sup>2</sup> MATTHEWS, S. A. : Journal of the American Medical Association, 1903, xli, p. 565.

<sup>3</sup> MCCALLUM and VOEGTLIN : Johns Hopkins Bulletin, 1908 (March), p. 91.

Since by asphyxiation results are obtained similar to the results obtained by the injection of salt solutions under the conditions discussed above, it would seem that this factor should constantly be borne in mind in interpreting the results obtained by the injection of such solutions.

## DIMINISHED MUSCULAR ACTIVITY AND PROTEIN METABOLISM.

By PHILIP A. SHAFFER.

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THERE are to be found in the literature many reports of investigations concerning the effect of increased or excessive muscular activity upon the excretion of total nitrogen, and of individual products of protein metabolism;<sup>1</sup> but the possible effect of greatly diminished muscular activity has received little or no attention. It has been shown repeatedly that with sufficient food a moderate increase of exercise does not lead to any considerable increase in total nitrogen excretion; but it does not necessarily follow that an abnormally diminished muscular activity may not affect some of the products of metabolism found in the urine.

Some knowledge of the effect of a low degree of activity appeared desirable as a further basis for the interpretation of metabolism results from pathological individuals, who, as a rule, are either confined in bed or take comparatively little exercise. Experiments on normal subjects were accordingly planned with a view of showing any change in the protein metabolism which might be caused by a decreased activity. "Work periods" were also included in each experiment, but the work was in no instance severe, and the results show the effect of only moderate activity. The subjects of the experiments were young men in good health.

The experiment with O. T. was divided into three periods, one which was spent almost continually in bed and at as far as possible complete rest, a second period during which the subject did his accustomed amount of work, and a third period during which he took additional but not excessive exercise. The period of accustomed work may be considered the "normal period," while the "rest

<sup>1</sup> Subject reviewed by MAGNUS-LEVY in VON NOORDEN'S *Handbuch der Pathologie des Stoffwechsels*, Berlin, 1906.

TABLE I.

## DIETS USED IN EXPERIMENTS.

I. O. T. <i>Rest Period.</i>		Nitrogen.	Calories.
Grain rice, 60 gm. . . . .		0.86	210
Quaker puffed rice, 25 gm. . . . .		0.32	190
Quaker rolled oats, 20 gm. . . . .		0.45	
Uneeda biscuit, 60 gm. . . . .		0.87	250
Eggs (whole), 100 gm. . . . .		1.92	140
Cream (18-20 per cent fat), 225 c.c. . . . .		0.90	450
Butter, 50 gm. . . . .		0.08	400
Milk, 100 c.c. . . . .		0.51	72
Sugar, 125 gm. . . . .		...	513
Salt, 5 gm. . . . .		...	....
One apple $\pm$ 150 gm. . . . .		...	75
		5.9 gm.	2300
II. O. T. <i>Normal Period.</i>			
Same as "Rest Period" with the addition of 50 gm. butter and 75 gm. sugar.			
Total Nitrogen, 6.0 gm.		Total Calories, 3000.	
III. O. T. <i>Work Period.</i>			
Same as "Normal Period" except 325 c.c. cream instead of 225 c.c. cream + 100 c.c. milk; 20 gm. cornstarch added.			
Total Nitrogen, 5.9 gm.		Total Calories, 3200.	
IV. O. T. <i>Non-purin.</i>			
Free mixed diet, excluding meat and meat soups. Subject ate many eggs, much milk, bread, cheese, etc.			
V. O. T. <i>Purin.</i>			
Subject ate a mixed diet, containing meat, at each meal. During periods IV and V no attempt was made to control the diets beyond the points stated.			
R. A. H. <i>First two days.</i>		Nitrogen.	Calories.
Quaker puffed rice, 56 gm. . . . .		0.78	195
Butter-thin crackers, 95 gm. . . . .		1.2	400
Butter, 45 gm. . . . .		0.07	360
Cane sugar, 90 gm. . . . .		...	370
Eggs (whole), 150 gm. . . . .		2.9	210
Milk, 1100 c.c. . . . .		5.8	790
Graham crackers, 45 gm. . . . .		0.5	190
		11.25	2515
R. A. H. <i>Rest of experiment.</i>			
Same as above, except 170 gm. sugar; 85 gm. puffed rice; 75 gm. butter.			
Total Nitrogen = 11.7 gm.		Total Calories, 3190.	



period " corresponds in amount of muscular activity to the condition of patients who are confined in bed and not unusually restless. The difference in the amount of muscular energy expended was obviously much greater between the rest and normal periods than between the normal and work periods.

In this experiment the rest period lasted for six days, two of which were spent wholly in bed, while on the remaining four the subject reclined quietly for a few hours each day in a Morris chair, the remainder of each day being spent in bed. The rest was so marked and prolonged as noticeably to weaken the subject, and in order to obviate any objection on this ground the rest period with R. A. H. was made only two days, which were spent wholly in bed. The experiment with R. A. H. was conducted somewhat differently in that, although the amount of muscular activity on the several days was so regulated as to make them quite different, the periods were not so sharply defined as in the other experiment.

The diets are given on page 446. That taken by O. T. was a low-protein diet, while that taken by R. A. H. contained about 11 gm. of nitrogen. Additional calories were supplied O. T. during the work periods as noted in the tables. These diets were constant except for the changes noted in the tables. After the close of the work period O. T. took a mixed high-protein non-purin diet for four days and then a diet containing much meat for six days. This change of diet was planned to obtain further data regarding the influence of meat and non-meat, and high-protein versus low-protein diets upon the composition of the urine. The urines were collected in twenty-four hour quantities.

The analytical results are given in Tables II and III. The analytical methods used are: Kjeldahl-Gunning for total nitrogen; Folin for urea, kreatinin, kreatin, and the sulphurs;<sup>2</sup> Folin-Shaffer for uric acid, and Boussingault-Shaffer for ammonia.

The conditions of these two experiments appear to me well adapted for the demonstration of any effect upon the protein metabolism (as indicated by the metabolic products of the urine), which might be caused either by a great decrease or by a marked though not excessive increase in the amount of muscular energy expended by the subjects. But on inspecting the results in the two tables we find absolutely no marked differences in the excretion of any metabolic product which can be explained by the variations in the amount

<sup>2</sup> FOLIN: *Journal of biological chemistry*, 1906, i, p. 131.

TABLE II (O. T.).

No.	Date, '06.	Volume.	Nitrogen as						Percent of total nitrogen.				Sulphur as			
			Total.	NH <sub>3</sub>	Kreatinin.	Kreatin.	Uric acid.	Rest.	Urea.	NH <sub>3</sub>	Kreatinin.	Rest.	Total.	Inorganic.	Ethereal.	Neutral.
1	Sept. 11	1625	5.81	0.29	0.66	...	0.13	0.30	76.3	5.0	11.3	5.2	0.435	0.274	0.030	0.131
2	12	1170	5.08	0.34	0.61	...	0.12	0.39	71.2	6.8	11.9	7.7	0.469	0.297	0.045	0.127
3	13	1330	4.82	0.35	0.60	...	0.10	0.31	71.8	7.2	12.4	6.4	0.451	0.297	0.043	0.111
4	14	1015	4.38	0.38	0.59	...	0.11	0.36	67.2	8.6	13.4	8.2	0.423	0.275	0.029	0.119
5	15	1160	4.29	0.37	0.58	...	0.11	0.36	66.9	8.6	13.6	8.4	0.428	0.252	0.030	0.146
6	16	830	4.26	0.36	0.59	...	0.11	0.38	66.3	8.4	13.8	8.9	0.421	0.272	0.039	0.110
	Average ..		4.77	0.35	0.605	...	0.11	0.35	70.5	7.3	12.7	7.2	0.438	0.278	0.036	0.124
7	17	1070	4.85	0.42	0.58	...	0.12	0.41	68.6	8.7	11.9	8.5	0.422	0.267	0.031	0.124
8	18	705	4.50	0.38	0.60	...	0.09	0.42	66.9	8.4	13.4	9.3	0.427	0.271	0.047	0.109
9	19	720	4.13	0.37	0.57	...	0.10	0.42	64.6	8.9	13.8	10.2	0.404	0.259	0.046	0.099
10	20	735	4.12	0.35	0.65	...	0.11	0.42	62.9	8.4	15.7	10.2	0.420	0.274	0.041	0.105
11	21	1170	4.01	0.36	0.61	...	0.11	0.43	62.4	8.9	15.1	10.7	0.407	0.254	0.042	0.111
	Average ..		4.40	0.38	0.60	...	0.108	0.42	64.1	8.6	13.6	9.3	0.424	0.265	0.049	0.110
12	22	1260	3.78	0.36	0.65	...	0.11	0.37	60.6	9.6	17.1	9.8	0.407	0.261	0.045	0.101
13	23	1400	3.96	0.46	0.60	...	0.12	0.39	60.5	11.6	15.2	9.8	0.397	0.254	0.042	0.101
14	24	1180	3.43	0.44	0.54	...	0.12	0.39	56.6	12.9	15.6	11.4	0.398	0.252	0.039	0.107
15	25	1300	4.58	0.43	0.52	...	0.13	0.51	65.4	9.4	11.2	11.2	0.456	0.304	0.059	0.093
	Average ..		3.94	0.42	0.56	...	0.12	0.42	60.9	10.6	14.2	11.3	0.414	0.268	0.046	0.100
16	26	870	6.62	0.41	0.61	...	0.14	0.66	72.6	6.1	9.2	10.0	0.634	0.461	0.059	0.114
17	27	1610	11.82	0.69	0.56	...	0.10	0.81	81.7	5.8	4.7	6.8	0.889	0.680	0.070	0.139
18	28	1955	13.25	0.83	0.62	...	0.11	0.69	83.1	6.3	4.7	5.2	1.022	0.795	0.071	0.156
19	29	1380	12.40	0.73	0.58	...	0.10	0.94	81.1	5.9	4.7	7.5	0.962	0.750	0.070	0.142
	Average ..		11.02	0.87	0.59	...	0.11	0.775	80.5	6.0	5.3	7.2	0.877	0.646	0.068	0.138

TABLE II (O. T.).

Per cent of total S.			Total S $\times$ 100 Total N	Kreatinin.	Weight.	Mg. kreatinin per kg. body weight.	Remarks (for diets see p. 446).
Inorganic.	Ethereal.	Neutral.					
63.0	6.9	30.1	7.5	1.76	67.0		About 4 hrs. each day spent reclining in Morris chair. Rest of time in bed. <div>I. Rest Period.</div>
63.5	9.6	27.1	9.2	1.63			
65.9	9.8	24.3	9.3	1.62			
65.0	6.9	28.1	9.7	1.58			
58.9	7.0	34.1	10.0	1.57			
64.5	9.4	26.1	9.9	1.58	67.9		Whole time spent in bed. <div>I. Rest Period.</div>
<b>63.5</b>	<b>8.2</b>	<b>28.3</b>	<b>9.2</b>	<b>1.62</b>		<b>24.0</b>	
63.3	7.4	29.3	8.7	1.56	67.8		Laboratory work, but only little other activity. <div>II. Normal Period.</div>
63.5	11.0	25.5	9.5	1.62			
64.1	11.5	24.4	9.8	1.54			
65.3	9.7	25.0	10.2	1.75			
62.4	10.3	27.3	10.1	1.63	68.8		
<b>62.5</b>	<b>11.6</b>	<b>25.9</b>	<b>9.6</b>	<b>1.62</b>		<b>23.7</b>	Laboratory work + 10-mile walk. "Setting-up exercises" + 3½-mile walk. Walked 9½ miles + 5 hrs. hard work. Laboratory work + rapid 10-mile walk. <div>III. Work Period.</div>
64.0	11.0	25.0	10.8	1.74	68.8		
64.0	10.5	25.5	10.0	1.61			
63.3	9.7	27.0	11.6	1.45			
66.6	13.0	20.4	10.0	1.39			
<b>64.9</b>	<b>10.9</b>	<b>24.2</b>	<b>10.5</b>	<b>1.55</b>		<b>22.5</b>	Normal activity. <div>IV. Normal Period (higher protein, non-purin diet).</div>
72.5	9.5	18.0	9.6	1.64	69.1		
76.5	4.8	18.7	7.5	1.51			
77.8	7.0	15.2	7.7	1.68			
78.0	7.8	14.8	7.7	1.57			
<b>73.8</b>	<b>7.8</b>	<b>15.7</b>	<b>7.95</b>	<b>1.60</b>		<b>23.2</b>	

TABLE II (Continued).

No.	Date, '06.	Volume. c. c.	Nitrogen as						Per cent of total nitrogen.				Sulphur as			
			Total.	NH <sub>3</sub>	Kreatinin.	Kreatin.	Uric acid.	Rest.	Urea.	NH <sub>3</sub>	Kreatinin.	Rest.	Total.	Inorganic	Ethereal.	Neutral.
20	Sept. 30	1300	14.00	0.53	0.60	0.05	0.18	0.84	84.2	3.8	4.3	6.0	1.035	0.840	0.073	0.122
21	Oct. 1	2085	15.00	0.65	0.62	...	0.23	0.94	83.9	4.4	4.1	6.1	0.907	0.660	0.072	0.176
22	2	1780	15.20	0.65	0.65	...	0.21	0.70	85.6	4.3	4.3	4.6	1.172	0.918	0.068	0.186
23	3	1550	14.90	0.68	0.59	0.07	0.20	0.71	84.9	4.6	3.9	4.7	1.071	0.844	0.066	0.161
24	4	1860	14.88	0.54	0.65	0.07	0.20	0.85	84.6	3.7	4.3	5.7	1.077	0.863	0.073	0.141
25	5	1400	15.40	0.72	0.62	0.01	0.18	0.87	84.3	4.7	4.0	5.6	1.067	0.815	0.103	0.149
			14.90	0.63	0.62	0.03	0.20	0.82	84.3	4.2	4.2	5.8	1.055	0.823	0.076	0.156

of muscular activity. There are slight differences, to be sure, but they are in no case great enough to justify the conclusion that the amount of muscular activity is responsible. At the bottom of each table will be found the averages of the three periods; an inspection of these averages fully bears out the above statement. There are, however, some points of considerable interest which must be referred to.

A much discussed question in connection with the effect of muscular work has been its effect on kreatinin excretion, and it was largely my interest in this question which suggested these experiments.

In my experiments upon a patient with a permanent biliary fistula<sup>3</sup> and in many other yet unpublished experiments on diseased individuals, I have found a greatly decreased kreatinin excretion, which was increased when the patients improved sufficiently to take more exercise. The conclusion was therefore tentatively held that the diminished kreatinin excretion was a result of diminished muscular activity. In the meanwhile there appeared the paper of Hoogenhuyze and Verploegh<sup>4</sup> in which they conclude that with

<sup>3</sup> SHAFFER: This journal, 1906, xvii, p. 362.

<sup>4</sup> HOOGENHUYZE and VERPLOEGH: Zeitschrift für physiologische Chemie, 1905, xlii, p. 415. This paper contains a review of the literature.

TABLE II (Continued).

Per cent of total S.			Total S X 100 Total N	Kreatinin.	Weight.	Mg. kreatinin per kg. body weight.	Remarks (for diets see p. 446).
Inorganic.	Ethereal.	Neutral.					
81.1	7.1	11.8	7.4	gm. 1.61	kg. ...		} Normal activity . . . . . V. Normal Period (high protein, purin diet).
73.0	7.6	19.4	6.0	1.67	...		
78.3	5.9	15.8	7.7	1.74	...		
78.6	6.4	15.0	7.2	1.58	...		
80.3	6.6	13.1	7.2	1.74	...		
76.5	9.5	14.0	6.9	1.67	...		
78.0	7.2	14.8	7.1	1.67	...	24.2	

sufficient food muscular activity has no effect upon kreatinin excretion. However, in view of my results from pathological individuals it seemed to me possible that their negative conclusions could be explained by there being in their experiments perhaps no great difference between the amount of energy expended on the control days and that expended on the work days.<sup>5</sup> Whether or not this is a just criticism, it was decided to further test the question.

For reasons stated I fully expected to find a decreased kreatinin excretion in the subjects of my experiments during the rest periods. But the results show nothing of the sort. I therefore agree with the conclusion of Hoogenhuyze and Verploegh, as well as of certain earlier investigators, that muscular activity with adequate food has *per se* no effect on the excretion of kreatinin, which in these experiments is remarkably constant and quite independent of both the amount of food protein (Folin) and of the amount of muscular activity.

<sup>5</sup> The observers experimented upon themselves. They did each day their accustomed amount of laboratory work and on stated days took bicycle rides and gymnastic exercises. On account of the common inclination to remain quiet after marked fatigue it seems not unlikely that after their vigorous exercises the subjects may have rested for some hours, thus tending to reduce the difference in muscular activity between the control days and the work days.

TABLE III. (R. A. H.).

Twenty-four hours ending 8 A. M.	Volume.	Nitrogen.					Per cent of total nitrogen.			Sulphur as			
		Total.	Ammonia.	Kreatinin.	Uric acid.	Rest.	Urea.	Ammonia.	Rest.	Total.	Inorganic.	Ethereal.	Neutral.
Sept. '06.													
22	860	8.57	0.214	0.54	0.135	0.39	85.0	2.5	4.5	0.611	0.459	0.059	0.093
23	600	8.05	0.267	0.46	0.143	0.56	82.2	3.3	6.9	0.671	0.520	0.046	0.105
24	740	9.30	0.285	0.49	0.128	0.54	84.5	3.1	5.7	0.775	0.611	0.049	0.115
25	690	9.72	0.276	0.45	0.127	0.46	86.6	2.8	4.7	0.734	0.576	0.054	0.104
26	795	11.04	0.42	0.47	0.103	0.56	85.9	3.8	5.1	0.796	0.623	0.057	0.116
27	710	9.63	0.38	0.46	0.127	0.42	85.5	3.9	4.4	....	....	....	....
28	690	10.46	0.40	0.48	0.147	0.61	84.4	3.8	5.9	....	....	....	....
29	710	9.34	0.41	0.48	0.123	0.52	83.6	4.4	5.5	0.695	0.529	0.051	0.115
30	1020	9.27	0.38	0.46	0.108	0.55	83.5	4.1	5.95	0.664	0.493	0.061	0.110
Oct. 1	670	8.51	0.44	0.47	0.124	0.56	81.7	5.2	6.6	0.668	0.507	0.045	0.116
Av. of 30 and 1....		8.89	0.41	0.465	0.116	0.555	82.7	4.6	6.25	0.666	0.500	0.053	0.113
Av. 23, 27, 28, 29 ..		9.37	0.386	0.47	0.135	0.53	84.0	4.1	5.6	0.683	0.524	0.049	0.110
Av. 22, 24, 25, 26 ..		9.66	0.30	0.487	0.123	0.487	85.5	3.1	5.0	0.729	0.567	0.055	0.107

TABLE III. (R. A. H.).

Per cent of total S.			Total S $\times$ 100 Total N.	Weight.	Kreatinin.	Mg. kreatinin per kg. body weight.	Remarks.
Inorganic.	Ethereal.	Neutral.					
75.0	9.7	15.3	7.1	kg. 60.0	gm. 1.45	24.2	Laboratory work. Walked 9 miles. 1 hr. work out-doors.
77.5	6.9	15.6	8.3	...	1.24	20.6	Laboratory work. Walked 1 mile. Little activity.
78.8	6.3	14.9	8.3	...	1.32	22.0	Walked 7 miles. 2 hrs. work out-doors. Diet increased (see p. 446).
78.5	7.4	14.1	7.6	...	1.21	20.2	Walked 2½ miles. 7 hrs. work out-doors.
78.2	7.2	14.6	7.2	57.8	1.28	22.2	Laboratory work. Walked 5 miles.
...	..	...	..	...	1.24	21.5	In bed 11 hrs. Very little activity.
...	..	...	..	...	1.28	22.2	Laboratory work, but little other activity.
76.1	7.3	16.5	7.4	...	1.30	22.5	Laboratory work, but little other activity.
74.3	9.2	16.6	7.2	58.0	1.23	21.2	Whole 24 hrs. in bed.
76.0	6.7	17.3	7.8	...	1.26	21.8	Whole 24 hrs. in bed.
75.0	7.95	17.0	7.5	58.0	1.245	21.5	Whole time spent at complete rest in bed.
76.7	7.2	16.1	7.3	± 58.0	1.265	21.7	Laboratory work. Somewhat less than normal activity.
77.7	7.6	14.7	7.5	± 58.5	1.315	22.15	Extra work. More than customary exercise.

It is worthy of notice how slight was the increase of kreatinin during the period with meat diet (Table II). The average for six days is only 0.05 gm. (1.67 gm. kreatinin) more than on the low-protein non-meat diet (1.62 gm.). According to Folin<sup>6</sup> and Klercker<sup>7</sup> it is only the kreatinin (*i. e.*, not the kreatin) in the food (meat) which increases the kreatinin of the urine, and therefore the effect of the character of the food upon kreatinin excretion will depend upon the amount of kreatinin contained in the food eaten. Kreatin was not present in the urine of the subjects of these experiments, except in small amounts during the meat diet of O. T.

In Table II (O. T.) the total nitrogen decreased with increased activity, while exactly the opposite is found with R. A. H. It is unlikely that the amount of activity is in either case responsible. R. A. H. was losing weight during the first part of the experiment, while O. T. gained in weight during the whole time of the low-protein diet, and was undoubtedly storing protein. The figures regarding urea and ammonia again confirm Folin's statements, that the percentage of total nitrogen represented by urea decreases with decrease of total nitrogen, and that the absolute amount of ammonia decreases while the ammonia percentage increases, with decrease of total nitrogen. This is shown very strikingly in Table II. Neither urea nor ammonia was affected by change in the amount of muscular activity.

The excretion of uric acid is, according to these experiments, wholly unaffected by a decreased muscular activity.<sup>8</sup> Regarding the relation of uric acid to food, Folin<sup>9</sup> has found it to be slightly increased with increase of total nitrogen. In Table II the uric acid nitrogen (with non-purin diet) is not higher with 11 gm. total nitrogen than it was with 4 gm. total nitrogen.

<sup>6</sup> FOLIN: Festschrift für Hammarsten, 1906, iii, Upsala.

<sup>7</sup> KLERCKER: Biochemische Zeitschrift, 1907, iii, p. 45.

<sup>8</sup> CATHCART, KENNAWAY, and LEATHES: Quarterly journal of medicine, 1908, i, p. 416, Oxford, show that a marked increase of muscular activity causes, during the work, a decreased excretion of uric acid, which is followed, soon after the work has ceased, by an increased excretion. Within the narrower limits of the amount of work, as in my experiments, the excretion of uric acid is not affected.

<sup>9</sup> FOLIN: This journal, 1905, xiii, p. 86. FOLIN's conclusion is somewhat contrary to that of BURIAN and SCHUR (Archiv für die gesammte Physiologie, 1900-1903, lxxx, lxxxvii, and xciv), and of SIVEN (Skandinavisches Archiv für Physiologie, 1901, xi), who found the endogenous excretion of uric acid to be constant.



There is no evidence that the undetermined nitrogen is affected in any way by the change in muscular activity. The differences are relatively slight and almost, if not quite, within the limit of error.

The figures in Table II confirm Folin's "law" concerning undetermined nitrogen.

The total sulphur runs parallel with the total nitrogen, the inorganic sulphur parallel with urea, and the ethereal sulphur practically parallel with ammonia. No one of the above is affected by muscular activity. In the case of neutral sulphur there is, in each experiment, a decrease with increase of muscular activity; but I do not conclude that the neutral sulphur always decreases with an increase of activity. Further experiments are necessary to decide this point. The neutral sulphur increases materially with increase of total sulphur, but not in proportion (Folin,<sup>10</sup> Table II).

The results of these experiments support the belief that with sufficient food either an increase or a decrease of muscular activity within physiological limits has *per se* no effect upon the protein metabolism as indicated by the nitrogen and sulphur partitions in the urine. We cannot, of course, believe that a long-continued diminished activity would not cause a change in the composition of the urine, because the intensity of metabolic processes in a muscle atrophied from disuse is certainly less than in a healthy muscle; but such a change in the composition of the urine should be considered not the direct result of decreased activity, but the result of a pathological condition, which, it may be, was brought about by a diminished activity. Exercise is necessary for health, but the amount of muscular energy expended in a given day (provided the amount is not excessive for the particular subject) does not appear to affect any of the nitrogenous substances of the urine excreted on that or following days.

I am indebted to two of my colleagues, Drs. Oscar Teague and R. A. Hatcher, who kindly consented to be the subjects of these experiments.

<sup>10</sup> SHAFFER: This journal, 1906, xvii, p. 375.

## SOME OBSERVATIONS ON THE NATURE OF HEAT PARALYSIS IN NERVOUS TISSUES.

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THE experiments summarized in this report were undertaken at the suggestion of Professor Carlson with the view of testing the asphyxia theory of heat paralysis in animal tissues. Some observations on the phenomena of heat paralysis in the heart tissues led Carlson to doubt the applicability of the theory to those phenomena, at least for the heart. There is no reason for believing that the mechanisms for heat paralysis in the heart tissues are different from those involved in the heat paralysis of the central nervous system and the peripheral nerves. If heat paralysis is due to lack of oxygen, recovery from heat paralysis should not be possible without the admission of oxygen. It may be stated at the outset that this is not borne out by the results of these experiments.

It has long been known that in certain cold-blooded animals, like the frog, a condition resembling motor paralysis could be induced by warming the animal until the internal temperature reached about 34° C. On cooling — provided the temperature went no higher and was not maintained too long — the animal recovered and in a short time was none the worse for his unusual experience. In the whole animal the condition of heat paralysis is pretty completely confined to the central nervous system, for at a time when the animal does not respond to stimuli applied to the skin the muscles respond to direct stimulation with an electric current; and if an isolated nerve trunk be stimulated, the muscles supplied by that nerve respond readily, but there are no movements in the other limbs. Thus all the reflexes through the cord are abolished at a time when the neuro-muscular mechanism is still able to conduct and respond to stimuli. Archangelsky proved the same point by allowing the leg

of a frog, separated from the animal except for the bone and nerve, to project from the warm chamber. In this animal, although the sensory end organs had been protected from the heat, he could elicit no reflexes by stimulation of the skin, although the reflex arc was intact so far as the afferent and efferent end organs were concerned (1). Heat paralysis is not confined to the central nervous system, for at certain temperatures the isolated nerve trunk loses its conductivity and excitability, only to regain both if the temperature is lowered. Heat paralysis is not a phenomenon confined to the nervous system. It appears in skeletal muscle as well, but here it possesses the peculiar characteristic of being a permanent paralysis from which no recovery can be made. A muscle when paralyzed by heating undergoes a slow shortening, and as soon as this contraction is well begun, no response can be elicited either by stimulation of the motor nerve or by direct stimulation with an electric current (2). It is thus apparent that the phenomenon of heat paralysis is not confined to the nervous system alone, but may be either in muscle or nerve. It must be admitted that we are probably dealing with different phenomena of heat paralysis in muscle and nerve, for in the nerve the heat paralysis is reversible, while in the muscle it is irreversible; in fact, it is plainly heat rigor. The case of the isolated heart presents the apparent paradox of a heat paralysis of muscle followed by a recovery on cooling. It must be remembered, however, that in the heart the muscular and nervous elements are so united that it is impossible for us to be certain upon which of the two anatomical elements the effect is produced. This is not true for the *Limulus* heart, where the paralysis of the heart ganglion and nerve plexus occurs at a lower temperature than the absolute paralysis and rigor of the heart muscle. It would seem probable, in view of the facts just stated in regard to the nature of heat paralysis of nerve and of muscle, that the recovery from heat paralysis, when recovery is possible, is due to recovery of the ganglion of the automaticity lost at the high temperature; and that the cardiac muscle was unaffected, for in this condition it can still be stimulated directly. The permanent standstill which appears if the organ be kept long at the temperature at which heat paralysis appears, or if it be raised a few degrees more, is a paralysis of the cardiac muscle itself, which from its very nature is an irreversible condition, from which no recovery could be expected.

That the condition of heat paralysis is not confined to cold-blooded

animals is proved by numerous researches. Eve (3), working on cats and rabbits, and using the condition of the pupil on stimulation of the cervical sympathetic as an index to the activity or paralysis of the superior cervical ganglion, found it possible to induce paralysis of that ganglion by warming to about  $50^{\circ}$  C. On cooling again the ganglion recovered its former activity. Alcock (4), using amphibia, animals, and birds, and making use of the negative variation as an index of the activity of the nerve, found that heat paralysis could be induced in the isolated nerve trunks of any of these animals, but that a higher temperature is necessary in those animals having a high temperature naturally — as is the case in mammals and birds — than in the amphibia.

Nor is the phenomenon of heat paralysis confined to the animal kingdom. A comparable condition is the inhibition of the protoplasmic streaming seen in cell of *Chara foetida* and *Nitella translucens*, when these plants are warmed to a temperature of  $55^{\circ}$ – $60^{\circ}$  C. If cooled at once, the plants show the streaming almost as vigorously as before. If the temperature is maintained for any considerable time, the streaming is permanently inhibited, or begins again feebly, and perhaps in the opposite direction. The recovery occurs without the admission of oxygen from without (5). Although it is not proved this is the same as the heat paralysis of animals, yet it must be admitted that it is a strikingly similar condition.

### I. EXPERIMENTS WITH THE WHOLE FROG.

The real point at issue in these experiments was to determine whether or not the higher centres have any connection with the spasmodic movements which appear when a normal frog is warmed to the temperature at which heat paralysis appears. The literature on this phase of the subject is not very extensive, and the views appear to be limited to two, — that of Winterstein, who believes that the spasmodic movements are due to a heat dyspnoea confined to the bulbar centres (6), and that of Carlson, who believes that the spasmodic movements are purposive movements on the part of the animal — originating in the higher centres — in a last attempt to escape from unpleasant surroundings (7).

In order to determine whether or not the higher centres were concerned, various parts of the brain were removed with anti-septic precautions from frogs under ether anæsthesia, and the ani-

mals placed in an aquarium for twenty-four hours to recover from the operation. Except in one or two cases only those animals which made a strong recovery and developed strong reflexes were used; the others were discarded.

For warming the frogs the following apparatus was used: The bottom of a wide-mouthed bottle, large enough to admit a large frog, was covered with moistened cotton. The bottle was closed with a rubber stopper provided with two holes. Through one was inserted a large glass tube to admit air, and through the other a thermometer which was pushed well down into the vessel. The bottle was then submerged in a large beaker of water into which steam was led from a large Erlenmeyer flask; with this apparatus the temperature of the air within the bottle could be raised very gradually and uniformly. Ordinarily about two hours were consumed in raising the temperature the twenty degrees necessary to induce heat paralysis.

To test whether or not heat paralysis had been established, the skin was stimulated with strong induction shocks. After the appearance of heat paralysis the internal temperature was taken, and the animal was cooled gradually. In some cases a second paralysis was induced in the same animal.

In testing whether or not the higher centres were concerned in the state of excitation which precedes the appearance of heat paralysis, four kinds of preparations were made: (1) cerebrum alone removed; (2) cerebrum and optic lobes removed; (3) transection just anterior to the medulla; (4) transection of the cord posterior to the cervical enlargement.

In animals prepared in the manner described in (1), (2), and (3) exactly similar results were obtained, when they were warmed in the same way, if they were in the same condition of recovery when the warming was begun. Thus the report of one experiment will show all which is to be learned from the three methods of preparation.

*Experiment 3. November 1.*—Large male frog, cerebrum removed October 31. Animal made an especially good recovery, respiratory movements normal. Animal maintained an almost normal position.

Observations made at five minute intervals, but most will be omitted.

Time.	Temperature.	Observations.
9.15	18° C.	Frog in normal position; breathing normal. Frog on the whole quiet. Moved around a little. Respiration practically normal.
10.30	30° C.	Respiratory rate increased.
10.45	33° C.	Respiration much increased; movements frequently occur.
10.55	34.5° C.	Violent leaping about in bottle.
11.00	35.0° C.	Violent movements, almost like tetanus.
11.05	36.0° C.	Strong extension of limbs, particularly of hind limbs.
11.06	36.5° C.	Animal collapsed; limbs extended. Heat paralysis.

The animal had lost its reflexes, although muscles respond to direct stimulation and to stimulation of the exposed sciatic nerve. Internal temperature, 33.5° C. Animal recovered on slow cooling and became as lively as before the first warming. A second warming later in the day gave similar results, and a second recovery was made. Autopsy revealed the complete removal of the cerebral lobes.

In cases where the animal did not recover well from the operation, although reflexes returned, the results were quite different.

*Experiment 8. November 8.* — Large female frog, cerebrum removed November 7, but the animal made poor recovery. Warmed in the usual manner.

Time.	Temperature.	Remarks.
8.00	18° C.	Frog motionless. Reflexes strong. No respiration.
8.15	20° C.	Frog motionless.
8.30	23° C.	
8.45	26° C.	
9.00	29° C.	
9.15	33° C.	
9.30	35° C.	Animal motionless.
9.45	39° C.	Animal toppled over in vessel. No movement.

Internal temperature, 34° C. Recovery very poor.

In the case of the transected cord I secured the movements of the forelimbs typical of the strong frog which had made a good recovery from the operation, but the hind limbs remained absolutely quiet. Not a movement or tremor was to be noted in them. Thus the normal course of events in animals with the brain removed to the

medulla during the induction of heat paralysis is: (1) A period of passive endurance interrupted by apparently voluntary movements of the limbs and changes of position until about  $33^{\circ}\text{C}$ . is reached in the warm chamber. (2) A period of violent movements ending in a spasm of a tetanic nature, attended by early violent respiratory movements followed by a gradual disappearance of respiration. (3) Complete paralysis; no movements of respiratory or voluntary muscles. Internal temperature,  $32^{\circ}$ – $36^{\circ}\text{C}$ . It should be noted here that during this stage the muscles respond to direct stimulation as well as to stimulation of the isolated nerve trunk, but no reflexes can be elicited from the opposite side, proving that the block must occur either in the centre or in the afferent path, — in all probability in the former, as the work of Archangelsky (1) cited above seems to show.

My observations are evidently confirmatory of Winterstein rather than of Carlson, inasmuch as the animals that were not in the condition of shock behaved in the manner described by him, and are confirmatory also of the view of the former that the medulla is the part affected by the increased temperature, inasmuch as the parts connected with the medulla always show the tetanic movements, while the parts separated from it do not show them at all. Animals in the condition of shock from removal of the brain down to the level of the medulla do not exhibit the excitatory stage prior to the paralysis. This is true even in cases of partial recovery from the shock to the extent of restoration of spinal reflexes. The absence of the excitatory stage in these animals is in all probability due to the depressed condition of the medullary centres.

The excitatory stage prior to heat paralysis resembles the excitatory stage of asphyxia, as pointed out by Winterstein. But this does not prove that the same causes are operative in the two phenomena. Moreover, the excitatory stage from heating is absent in the isolated spinal cord, while asphyxia produces convulsions even in the spinal animal.

## II. THE RELATION OF OXYGEN TO HEAT PARALYSIS.

This division of the article bears more directly upon the question of the nature of heat paralysis. The experiments show whether or not it is necessary to admit free oxygen to tissues in heat paralysis before recovery is possible, even if they be cooled to the temperature

at which they are ordinarily active. In other words, the problem is a test whether the heat paralysis is an asphyxia from lack of oxygen, as is claimed by Winterstein.

It is a very evident fact that the presence of oxygen is exceedingly essential to the continuation of life in an animal as a whole. But isolated parts of the body show an astonishing resistance to its absence in the gaseous form. Hermann (8) found in the case of muscle that although no oxygen could be removed from the muscle by means of an air pump, the tissue could maintain its activity for a long time in a medium devoid of oxygen. From this he concluded that muscular activity is practically independent of gaseous oxygen. Ewald (9), working on the motor nerves of frogs, found that if one sciatic nerve was placed in a vacuum, and the other from the same animal placed in a similar tube containing oxygen, there was no constant difference in regard to the time at which their activity ceased. Sometimes the one in oxygen remained active the longer, sometimes the one in the vacuum. In general, the activity continued for about seven hours. Similar results were obtained when one nerve was suspended in hydrogen and the other in oxygen. From his results he concluded that the activity of the nerve was independent of the supply of gaseous oxygen in the medium surrounding it. So far as reported, he made no test to find out whether or not the nerve suspended *in vacuo* or in the hydrogen recovered its activity on being removed to the air.

Winterstein (10), in his first work on heat paralysis, noted a similarity between the action of animals being paralyzed by heat and those asphyxiated in an atmosphere devoid of oxygen, and further stated that an animal paralyzed by heat would not recover if no oxygen was admitted to it, even if it be cooled down to the normal temperature. He noted recovery in an animal warmed in the incubator, a recovery which he ascribes to oxygen present in the blood, but no recovery was noted if the animals were perfused with boiled salt solution, thus removing all oxygen remaining. He also showed that heat paralysis appeared earlier in an animal poisoned with strychnine, — a drug which he claimed had no effect except to increase the metabolism of the body, thus hastening the disappearance of the oxygen from the tissues. He explained the process of heat paralysis with its foregoing phenomenon of higher excitability on the basis of the "biogen molecule," claiming that the dissimilation exceeds the assimilation and that finally a condition of exhaustion is reached.



Inasmuch as the animal does not recover until oxygen is admitted, he assumed that the substance the assimilation of which was delayed was oxygen. Thus he concluded heat paralysis must be an asphyxia from lack of available oxygen at high temperatures. The movements of the animal during the excitation stage are stated to be the result of increased dissimulation by the abnormally high temperature. Von Baeyer (11) found that the nerve cells of frogs contained organic material enough to suffice for nine hours, but that there was a constant demand for oxygen. He found that if an animal were paralyzed by driving out the blood with warm boiled NaCl solution and later revived by perfusing with a cold solution rich in oxygen, the animal would react longer than if the perfusion were performed with a warm solution. He claimed that this difference is due to the fact that at low temperatures the cells store oxygen in larger quantities than at higher temperatures. He believed that the oxygen was stored by entering into a somewhat loose chemical composition with some substance in the protoplasm which held it firmly at low, and loosely at higher, temperatures. Inasmuch as a cold strychnine frog responds less frequently to stimuli than one which is warmed, he believed that the oxygen leaves the reservoirs by diffusion, which, being a purely physical process, is governed directly by the temperature. He could not correlate this explanation with the observation that a frog reacts more readily to stimulation at low than at high temperatures, and was driven to the assumption that the cold renders the "explosive" substance more unstable than it is in the warmer temperatures. The higher excitability preceding heat paralysis he ascribed to the flooding of the centres with oxygen, the diffusibility of which was increased by the warming. The heat paralysis he explained as an exhaustion following the inability of the explosive substance to combine with oxygen and the inability of the reservoir to hold its oxygen any longer. He, too, considered heat paralysis an oxygen asphyxia, from which no recovery is possible without more oxygen from the air. Later Von Baeyer (12) extended his observations to the isolated nerve fibre. He found that a nerve suspended in an indifferent gas, hydrogen or nitrogen, remained active for a long time, but ultimately lost both irritability and conductivity, — functions which were recovered, according to his observations, only in the readmission of oxygen. He found that by raising the temperature, thus increasing the rate of diffusion, this condition of paralysis could be hastened very markedly. Further, the

use of reducing agents tended, by the removal of oxygen, to hasten the appearance of the paralysis, which he assumed to be identical with heat paralysis. Fröhlich (13) cited experiments to show that there are storehouses for oxygen present in the isolated nerve fibres similar to those shown by Von Baeyer to be present in the cord. Further, his work tended to show that the process by which the oxygen leaves the reservoir is one of pure diffusion, its rate being increased by heat and decreased by cold and entirely independent of the presence of anæsthetics. Bondy (14), working also with the nerve centres of frogs, shows the presence of oxygen storehouses from which the oxygen passes by a mere diffusion. He explains the higher excitability of the warmed frog to the flooding of the centres with oxygen, owing to the rapid diffusion at that temperature, — a condition which renders their discharge more easy, and the heat paralysis to the exhaustion of the store of oxygen available. Winterstein (15), after a careful study of the respiration of the isolated cord of the frog, could not determine any difference between the amount of oxygen taken in and that given off as carbon dioxide after a more or less complete asphyxia of the cord, and thus was not even convinced that such storehouses are present; nor could he venture any statement in regard to the manner in which the oxygen is held in the storehouse, or by what means it passes to the places where it is required. After all, it must be agreed that the presence of these storehouses is still more or less hypothetical, and in regard to the manner in which the oxygen is held, not even a guess can be hazarded. Winterstein (16), in a later work, after a careful analysis of the oxygen used by animals at various temperatures, showed that at higher temperatures, still beneath the point at which the paralysis appears, the rate at which oxygen is used is much increased, and also that even in animals in a condition of heat paralysis a certain amount of oxygen is still used. He claimed that by the use of anæsthetics the oxidative processes alone are hindered, and that the effect is exactly the same as that of the higher temperature which induces heat paralysis, namely, rendering the cell unable to use oxygen. As proof, he cites the appearance of heat paralysis earlier in a narcotized than in the normal animal, and the fact that an incomplete anæsthesia may be changed into a complete one by merely raising the temperature.

These writers seem to be unanimously of the opinion that heat paralysis can be due to only one thing, namely, an asphyxia, and

that recovery without the addition of oxygen from without is an impossibility. But certain characteristics of heat paralysis seem to render a different view possible, or at least render this view improbable.

**Experimental methods.** — In the consideration of the problem of the nature of heat paralysis, there is no reason for believing that the paralysis in one part of the nervous system differs qualitatively from that in any other part. For the sake of convenience of preparation, many of the experiments to be cited below were performed upon the isolated sciatic of the frog, and the contraction of gastrocnemius was used as an index of the activity of paralysis of the nerve. Other experiments of the same kind were performed on the isolated nerves of turtle, upon the cord of the turtle and frog, and upon the automatic ganglion of the *Limulus* heart. The object was to study the appearance of heat paralysis in the absence of oxygen, and to note whether or not recovery was possible without the addition of oxygen from the air.

1. It was thought at the beginning that the precaution used by the former investigators would be sufficient for the work at hand, so a number of experiments were run, immersing the nerve in boiled salt solution covered with castor oil to prevent so far as possible the return of oxygen to the water. It was soon found, however, that this method had to be discarded, for no matter how long the solution was boiled a positive test for oxygen could be secured with potassium hydrate and pyrogalllic acid, thus rendering the results worthless.

2. The next medium employed was paraffine oil. In a preliminary test run to ascertain whether or not the oil was toxic to the nerve it was found that after the nerves had been suspended in the oil for five hours and twenty minutes the muscle responded to stimulation just as effectively as in the beginning. This proved that the toxicity, if any existed, was too slight to make any corrections for the error necessary, especially since the time of an experiment was so brief. The use of this oil is open to criticism, inasmuch as it was shown in later tests that although the oil gave no test for oxygen with anhydrous potassium hydrate and pyrogalllic acid, nevertheless a small amount of oxygen was present in the oil. The addition of the KOH and pyrogalllic acid directly to the oil gave a negative test, probably because neither is soluble in the oil. If, however, water is boiled for four hours in a flask pro-

vided with reflex condenser, and then while boiling allowed to flow under the oil from a pipette, on the addition of KOH and pyrogalllic acid, a violet color appears throughout the water, heaviest at the top. Even if melted paraffine be poured upon the oil immediately, a dark ring from the point of junction of the oil and water shows the presence of oxygen in the oil which has diffused from it into the water below. Evidently, then, there was a small quantity of oxygen in the oil, which of course renders the results in that medium open to criticism, but the results are checked sufficiently by the experiments with hydrogen to render any very glaring error impossible. And furthermore, in many of the experiments, the oil was boiled vigorously before using, and in these the same results were secured as in those in which such strenuous measures were not adopted; and for this reason it is believed that even though a trace of oxygen was present, it was not present in quantities sufficient to invalidate the results secured by the methods. My experience here shows the difficulties which attend work with oxygen-free solutions. Winterstein, Bondy, Von Baeyer, and others in their perfusion experiments, used only boiled salt solutions which they cooled to the proper temperature. They assume that such solutions are oxygen free, and that such is not the case can be seen from the experiments cited above.

3. The third medium used was hydrogen. The gas was generated in a Kipp's apparatus from a pure zinc, hydrochloric acid, and copper sulphate. From the generator the gas passed through basic lead acetate solution before entering the chamber where the nerve was placed. Notwithstanding the work of Von Baeyer and Ewald, I considered it advisable to run preliminary tests to determine whether or not hydrogen was an indifferent gas to the nerve. The sciatics and gastrocnemius muscles of a large frog were isolated, and suspended in a moist chamber. One muscle was suspended by its femur, the nerve was passed through a gas chamber, and the central end laid across the electrodes. The other muscle was suspended in the same way, and its nerves laid across the same electrodes. The whole preparation was kept moist in air. The holes in the gas chamber through which the nerve passed were then blocked with kaolin, and a rapid stream of hydrogen passed through continuously.

The preparations were tested at various times at room temperature. The experiment was begun at 8.50. At 11.55 both nerves

were active; at 12.20 the nerve in air was inactive, but the one in hydrogen was still active. At 1.32 both were inactive and were removed from the electrodes. The nerve in gas looked dried and shrivelled. The preparations were wrapped in moist filter paper and tested for irritability. At 2.45 the nerve which had been in hydrogen responded weakly, but soon failed to respond. The recovery here was undoubtedly due to the restoration of moisture to the dried nerve. The other, which had been in the air, never recovered its activity. Another preliminary experiment was run, and in this case at the end of the observation — after four hours and twenty minutes — both nerves were still active. From these experiments I concluded that hydrogen was no more injurious to the nerve than the ordinary oxygen and nitrogen of the air, and that drying was the worst foe to be contended with in the experiments.

The apparatus used for inducing heat paralysis in oil was a very simple one. A loop of the nerve was fastened to the bottom of a large watch glass and covered with oil heated to the proper temperature. When heat block was established, cold oil was poured into the watch glass and the excess allowed to run over the sides into a large flat pan. In this way the nerve was never exposed to the air after the heating was begun. The stimulus to determine whether or not heat paralysis was established was an induced current applied near the end of the nerve. The possibility of the response being due to a spread of current was guarded against by tying off the nerve under the oil after the heat paralysis had been established and recovery effected. The failure of the muscle to respond after such a ligature had been applied showed conclusively that the recovery noted was not due to a spread of currents through the oil to still sensitive portions of the nerve beyond the point of heating. If such had been the case, the muscle would have responded as well after tying the ligature as it did before. Although the thermometer was always used, this method does not give an accurate idea of the temperature of the nerve; but this was no disadvantage in this case, since the temperature at which heat paralysis occurred was of no particular importance.

The apparatus for heating the hydrogen and exposing the nerve to it was the following: From the Kipp generator the gas was led into the stem of a Y tube, one limb of which connected with a tube which ended in the bottom of a wash bottle two-thirds full

of hot basic lead acetate, the other connected with a tube which ended in a similar manner in a wash bottle of cold basic lead acetate surrounded with freezing mixture. The wash bottles in both cases had been filled full while the solution was boiling and then about one-third of the liquid displaced with hydrogen. This renders the amount of oxygen present practically negligible. From the wash bottles the gas was led by glass tubes to the limbs of a Y tube, the base of which was connected with the inlet of the gas chamber. By means of this system of tubes either hot or cold gas could be applied to the nerve at will. The gas chamber was made from an ordinary large T tube. The stem of the T was fitted with a rubber cork through which a thermometer was admitted. An entrance tube was sealed on below, and an exit tube, fitted with a bent portion to end under water, was sealed on above, one on either side of the stem of the T. The ends of the bar of the T were rounded down until the opening was only 2 mm. across. Through this chamber the nerve passed from end to end, and hot or cold gas could be admitted at will. The nerve was drawn through with a string, and sealed into position with kaolin. After the nerve was placed in position at the beginning of the experiment, the air was displaced by a rapid stream of cold hydrogen until the gas burned without explosion, and throughout the experiments gas bubbled through the system continuously, thus insuring a positive pressure within, and revealing at once any leak in the apparatus, all of which occurred at the point where the nerve was sealed into the gas chamber with the kaolin.

**A. Results in the isolated nerves.** — The experiments of this kind were made upon the sciatic nerves of frogs in both hydrogen and oil, and upon nerves from turtles in oil. The results are exactly alike, so the error due to the presence of traces of oxygen in the oil cannot be a factor.

The results secured in experiments in oil on isolated sciatic nerve of frog were the same throughout the work. The following experiments carried out January 21, 1908, are typical:

*Experiment 1.* — Oil applied in the usual way and paralysis was secured, but no recovery ever occurred, although the nerve was removed to the air, wrapped in moist filter paper, and watched. Probably killed by warming to too high a temperature.

*Experiment 2.* — Heat paralysis and recovery secured in oil. No response in the muscle after ligation of the nerve under the oil.

*Experiment 3.* — Heat paralysis and recovery. Experiment repeated and the ligature was tied after the second heating and recovery.

No response elicited by strong stimulation after tying the ligature.

*Experiment 4.* — Repetition of Experiment 3.

*Experiment 5.* — Heat paralysis and recovery secured three times. No response after the application of the ligature.

*Experiment 6.* — Heat paralysis and recovery secured four times. No response after the application of the ligature.

Temperature of heat paralysis about  $41^{\circ}$ – $42^{\circ}$  C.; of recovery, about  $38^{\circ}$ – $39^{\circ}$  C.

The results secured with the isolated nerves of frogs in an atmosphere of hydrogen were similar to those cited above. The hydrogen was generated and applied as described. The following experiments carried out February 22, 1908, are typical:

*Experiment 3.* — Heat block and recovery twice in hydrogen. Nerve died suddenly after second heating, and never recovered its activity, although it was removed to air and closely watched.

*Experiment 4.* — Heat paralysis and recovery four times. Died after fourth recovery.

*Experiment 5.* — Heat paralysis and recovery twice. Died after second recovery.

*Experiment 6.* — Heat paralysis and recovery twice. Died after second recovery.

*Experiment 7.* — Heat paralysis and recovery four times. Died after fourth recovery.

*Experiment 8.* — Killed during first heating.

*Experiment 9.* — Killed during first heating.

*Experiment 10.* — Heat paralysis and recovery once. Killed during second heating.

*Experiment 11.* — Heat paralysis and recovery seven times. Killed during eighth heating.

Temperature of heat paralysis about  $33^{\circ}$  C. It was observed that the temperature as heat paralysis appeared was practically the same for consecutive experiments in the same preparation, although it varied slightly in different animals.

In the work on the isolated nerves of turtles the muscles of the hind limbs were used as the indicator of the activity of the nerve. In general the nerves of these animals appear more resistant to heat paralysis than those of frogs. The following experiments on a single preparation in oil are typical:

**Preparation No. 1.**

*Experiment (a).* — Heat paralysis established at 40° C. The temperature rose to 43° C. before it could be lowered by cool oil. Recovery was perfect.

*Experiment (b).* — Heat paralysis at 43° C. Temperature rose to 45° C. Recovery was perfect.

*Experiment (c)* — Heat paralysis at 43° C. Temperature rose to 44.5° C. Recovery was perfect.

As was stated before, the temperature cannot be taken accurately by this method, inasmuch as insufficient time elapses to permit the preparation to reach throughout the temperature of the medium surrounding it.

In all of these experiments, even in those in which the paralysis by heat was secured as many as seven times, no oxygen was admitted after the first heating was begun. In the case of the frog's nerves in hydrogen some peculiar facts are to be noted; the limits between full activity, heat paralysis, and heat death are exceedingly narrow at high temperatures, as was noted by Eve (3) for low temperatures.

Heat paralysis can be secured in nerves practically instantaneously merely by heating the preparation to the proper temperature, and recovery can be secured practically as quickly by merely lowering the temperature. *No oxygen need be admitted from without.*

**B. Results on the isolated cord.** — The next point to determine was whether or not the cord behaved in a manner similar to the nerve fibre in its relation to oxygen. The fact that the "synapse" is brought in here might possibly modify the result. That such was not the case is shown by the following experiment:

The brain of a large frog was pithed and the cord laid bare together with the sciatic and the gastrocnemius of both sides. The lumbar enlargement, together with the upper ends of the sciatics, was fastened to the watch glass as the nerves had been in the previous experiments. The ventral surface of the cervical portion of the cord was laid on the electrode. Both muscles responded to stimulation. Oil warmed to 38° C. was poured over the preparation, submerging it completely, excepting the muscles and the portion of the cord on the electrodes. Heat paralysis was established. The preparation was then cooled by pouring in cold oil, and recovery occurred. As a precaution to guard against a spread of current, the nerves were ligated under the oil. On further stimulation no



response could be secured. Other preparations gave exactly the same results.

The cord of the turtle, since it was so much larger than that of the frog, seemed better adapted for the experiments. The animal was decapitated, and in some cases two preparations were made from the same animal, — the lumbar enlargement with lumbo-sacral plexus and hind limbs serving as one, and the cervical enlargement with the brachial plexus and the forelimbs as the other. The following experiment shows the result, secured from the latter preparation :

*February 3, 1908.* — After decapitation the cord of the animal was transected immediately behind the cervical enlargement; the shell was cut away, and the whole cervical enlargement and the brachial plexus were dissected out and placed in a flat dish. The whole cervical enlargement was then immersed in oil. The cord was stimulated in the neck region, at least two inches from the oil. Heat paralysis and recovery were secured ten times, and after the tenth recovery the preparation responded as actively as at the beginning. At no time during the entire course of the experiment was oxygen admitted from without, as the preparation was kept entirely submerged in the oil.

In order to confirm the work on the cord in oil, the behavior of the cord in hydrogen was tested. The cord of a small turtle was isolated and drawn through a gas chamber as the nerve of the frog had been in the former experiment. Warm hydrogen was admitted and heat paralysis established, — a condition which was replaced by activity as soon as the cold hydrogen was admitted. As many as three paralyzes and recoveries were secured from a single preparation, and in this case the preparation died, as the frog nerves had done, before warm hydrogen could be admitted for the fourth time.

From the results above — those on the frog's cord in oil, the turtle's cord in oil, and the turtle's cord in hydrogen — it is apparent that even where a synapse is concerned, heat paralysis may be established by raising the temperature, and recovery secured merely by lowering the temperature *without the admission of oxygen from the air*. For this reason heat paralysis is not due to a lack of oxygen, for, if such were the case, no recovery could be made unless oxygen were admitted from without to supply the tissues affected by the heat.

C. **Results on the heart of the *Limulus*.** — The tests were made only in hydrogen. The heart was removed from the animal in the usual manner. In some cases the median dorsal ganglion upon which the automaticity of the heart is due (17) was dissected free from the muscle, except in the anterior portions of the organ. The ganglion was then put into the gas chamber and sealed in with kaolin. In other cases the heart was simply removed from the animal and the posterior two-thirds introduced into the chamber. The contractions from this sort of a preparation are much stronger than from the first preparation and the results are exactly the same. Heat paralysis was induced and recovery noticed. The following work done July 2, 1908, shows the results sufficiently well:

*Preparation No. 1.* — Heat paralysis and recovery induced twice. Preparation killed on the third warming.

*Preparation No. 2.* — Heat paralysis and recovery eight times; killed on ninth warming.

*Preparation No. 3.* — Heat paralysis and recovery six times; killed on seventh warming.

*Preparation No. 4.* — Heat paralysis and recovery once; killed on second warming.

*Preparation No. 5.* — Preparation killed on first warming.

It will be noted that in all these preparations death occurred during one of the processes of warming, and in not one of these cases did recovery occur on removal to moist filter paper, although all were carefully watched and tested. So far as can be seen no error can be claimed in this set of experiments. There can be no oxygen present in the gas chamber, and there can be no spread of current, inasmuch as the contractions arise from impulses originated by the automatic ganglion in the normal manner. In four of the preparations paralysis and recovery were secured in the total absence of oxygen, in one, as often as eight consecutive times.

While the experiments of Winterstein, Von Baeyer, Bondy, and others are very clever, it does not seem that their results — being negative as compared with the positive results secured in the isolated nerve, isolated cord, and the *Limulus* heart ganglion — are conclusive proof of the claim that the paralysis due to heat is an oxygen asphyxia. The fact that an animal while being warmed executes movements similar to one being asphyxiated in nitrogen does not mean that those movements are due to the same cause.

And the fact that the animal rendered motionless by either heat or asphyxia does not recover until oxygen is admitted does not prove, as Winterstein would have us believe, that the effect is merely an asphyxia of the ganglion cells by a lack of oxygen. The perfusion experiments of Winterstein and others are too radical not to involve other changes of as much importance as a lack of oxygen. The use of strychnine, alcohol, and other substances is to be criticised, also, as involving changes more far-reaching than a mere increased metabolism and a decreased oxidation, — the only effect the investigators claim for them. An asphyxia does not explain why it is that an animal in so-called "poor condition," arising from too long a confinement under unnatural conditions of medium and temperature, is paralyzed by heat at a temperature so much lower than the normal animal just removed from its natural environment, and yet such is admitted to be the case. Nor does the asphyxia theory account for the fact that in the vertebrate the heat paralysis of the ventricle occurs at a lower temperature than that of the auricle and sinus venosus, and that tension in the heart cavities raises the temperature required for the paralysis. In the *Limulus* heart the muscle ceases to respond to the impulses from the ganglion at temperatures under which it still responds to direct stimulation. This is a form of heat paralysis, but it cannot be due to lack of oxygen.

If the heat paralysis is due to asphyxia, granting the presence of oxygen reservoirs, how can the paralysis be secured when the reservoirs are full of oxygen, as must be the case in the freshly isolated cord and nerve? Yet the experiments cited in this paper show that the first paralysis occurs at the same temperature as any of the succeeding paralyses, neither higher nor lower so far as could be determined. It is to be expected, however, granting that the paralysis be due to an asphyxia, that the paralysis would occur at a lower temperature on the later paralyses than on the earlier ones, inasmuch as some of the oxygen surely would have been used up or would have diffused out, and thus less oxygen would have been available toward the end. These experiments prove conclusively that either heat paralysis occurred in the presence of oxygen, or else that recovery occurred in the absence of that gas, either case being sufficient to show that heat paralysis is not an asphyxia due to a lack of oxygen; and furthermore, the evidence of the actual presence of oxygen reservoirs is not conclu-

sive, as Winterstein (15) himself has shown. If there are no such things as reservoirs present, provided then that heat paralysis is due to an oxygen asphyxia, from what source does the cell get its new supply of oxygen with which to resume its activity after heat paralysis? It is exceedingly difficult to understand how, if heat paralysis is due to asphyxia, it can manifest itself as a practically instantaneous phenomenon. It came out clearly in the case of the isolated nerves and cord; and, furthermore, it has been noted in the case of the *Limulus* heart ganglion that merely raising the temperature a few degrees can accomplish what it takes ten to fifteen hours to accomplish in an atmosphere of pure hydrogen, namely, a paralysis from a lack of oxygen (7). This experiment cited by Carlson showed that there was oxygen sufficient in the ganglion to maintain its activity for hours, but if the temperature is raised the phenomenon occurs within the time required for a single discharge. Therefore, in my experiments in hydrogen heat paralysis either occurred in the presence of oxygen — for it seems exceedingly improbable that all the oxygen could have been used up or have escaped in so brief a time — or else, if all the oxygen was gone, recovery from the paralysis occurred in the total absence of oxygen. Either view renders the theory of asphyxia as an explanation of heat paralysis impossible.

It is difficult to explain the discrepancies in the results which exist between the experiments of Von Baeyer and my own. The methods were practically the same, and yet the results were entirely different. It is possible that we have observed totally different phenomena. It will be noticed that Von Baeyer's experiments were conducted at a temperature requiring from twenty to forty minutes after the beginning of the warming process for heat paralysis to appear. Thus more time was allowed for diffusion, and the paralysis he noted may have been a true oxygen asphyxia, from which recovery is possible on the readmission of oxygen. The paralysis dealt with in the experiments cited above and which certainly is the true heat paralysis is practically instantaneous in its appearance, and disappearance lies between exceedingly narrow limits close to the thermal death point of the tissue, and is independent of the presence or absence of oxygen.

In addition to the evidence above we have the observations of Babàk (18), who found that the *rana esculenta* was exceedingly resistant to heat paralysis but easily paralyzed by a lack of oxygen,

while the *rana fusca* was resistant to a lack of oxygen but succumbs readily to heat paralysis. Amerling (19) confirmed the observations made by Bakák by his work on the developing frog.

Since the resistance to asphyxia and heat reveals such marked differences in these two kinds of frogs, it seems exceedingly probable that other and greater processes than a lack of oxygen are involved in heat paralysis.

#### SUMMARY.

To recapitulate briefly, the facts established in this paper are the following:

1. The movements during period of excitability in the whole frog preceding the appearance of heat paralysis are independent of the higher centres and originate in the medulla.
2. In animals in a condition of shock from removal of the brain down to the level of the medulla the period of excitability prior to the onset of heat paralysis is not in evidence.
3. It is possible to establish a heat paralysis and secure a recovery in the isolated nerve and in the isolated cord of frogs and turtles, and in the automatic ganglion of the *Limulus* heart without admitting oxygen from the air. This process may be repeated a number of times on the same preparation in the complete absence of oxygen.
4. In view of this fact it seems necessary to conclude that heat paralysis is not due to an asphyxia from a lack of oxygen.

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12. VON BAEYER, H. : *Ibid.*, 1902-1903, ii, p. 169.
13. FRÖHLICH : *Ibid.*, 1903-1904, iii, p. 75.
14. BONDY, O. : *Ibid.*, 1903-1904, iii, p. 180.
15. WINTERSTEIN, H. : Centralblatt für Physiologie, 1906, xx, p. 41.
16. WINTERSTEIN, H. : Zeitschrift für allgemeine Physiologie, 1905, v, p. 323.
17. CARLSON : This journal, 1904, xii, p. 67 ; 1905, xii, p. 471.
18. BABAK : Centralblatt für Physiologie, 1907, xxi, p. 6.
19. AMERLING : Archiv für die gesammte Physiologie, 1907, cxxi, p. 363.

## THE APPLICATION OF McDOUGALL'S THEORY OF CONTRACTION TO SMOOTH MUSCLE.

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NO one who has made a special study of the subject of muscular contraction can fail to be impressed by the difference in the amount of attention received by the two great classes of contractile tissue. In the physiological textbooks striated muscle receives five times as much space as smooth muscle; while the numerous theories of contraction either ignore muscular structure altogether or concern themselves exclusively with the structure of striated muscle. And yet smooth muscle is the more primitive and widely distributed form, and it is reasonable to suppose that its contractile machinery is simpler than that of its more energetic sister tissue.

In a recent article<sup>1</sup> I have reported a number of observations which confirm the already highly probable hypothesis regarding the contraction of striated muscle put forward by McDougall<sup>2</sup> about ten years ago.

McDougall's hypothesis is, in brief, that the sarcostyles or fibrillæ of striated muscle possess a structure of such nature that shortening is the necessary mechanical result of their distention; and, further, that contraction in this form of muscle is ordinarily the result of distention of the sarcostyles caused by their absorption of a part of the sarcoplasmic fluid which surrounds them. This hypothesis receives, as has been said, much support from a number of facts concerning the histology and physiology of striated muscle. But any "theory of contraction" must remain unsatisfactory so long as it applies only to the lesser half of the subject; and to any one who hopes that McDougall may have found a guiding thread

<sup>1</sup> MEIGS: *Zeitschrift für allgemeine Physiologie*, 1908, viii, p. 81.

<sup>2</sup> McDOUGALL: *Journal of anatomy and physiology*, 1897, xxxi, p. 410; 1898, xxxii, p. 187.

through the confused region of muscular contractility, two questions immediately present themselves: Is there to be found in smooth muscle any element comparable to the sarcostyle of striated muscle? Is there any reason to believe that in smooth muscle also the process which may be called *imbibition* is the constant accompaniment and underlying cause of contraction? The following article attempts to begin the answering of these two questions.

### I. THE STRUCTURE OF SMOOTH MUSCLE.

A well-known recent review of the structure of smooth muscle is that of M. Heidenhain.<sup>3</sup> This author is inclined to believe that striated muscle and smooth muscle have an essentially similar structure. It is his opinion that the cells of the smooth muscle are made up of fibrillæ embedded in an interstitial substance exactly as are those of the striated muscle, and he even makes the suggestion (p. 212) that the smooth muscle fibrillæ are in reality cross-striated, like those of skeletal muscle. He believes that histologists may hitherto have failed to perceive the cross striations of involuntary muscle simply because microscopes and staining methods have only now been brought to a sufficient pitch of perfection.

That Heidenhain's opinions are more or less in agreement with those of other histologists is shown by the nomenclature of the subject. Both striated and smooth muscle are said to be made up of fibres, and the fibres, in turn, of fibrillæ. It is plain, however, that the resemblance between the two tissues, if it exists, is not a resemblance that appears on the surface or to the casual observer. Probably nobody with the least pretence to being a histologist ever mistook one tissue for the other, however poor his preparations may have been. On the other hand, there is a fairly close resemblance between smooth muscle and certain forms of connective tissue, and even well-trained histologists may be excused for occasionally making a mistake in this direction. The history of the subject is significant in this respect; before the year 1847 smooth muscle was known as "contractile connective tissue."<sup>4</sup>

The evidence from the embryology of the two tissues favors the views of the older histologists. Striated and smooth muscle are

<sup>3</sup> M. HEIDENHAIN: *Ergebnisse der Anatomie und Entwicklungsgeschichte* 1900, x, p. 115.

<sup>4</sup> FLEMMING: *Zeitschrift für Zoologie*, 1878, xxx, Supplement, p. 466.



different from the very beginning of their development and at every stage of the process,<sup>5</sup> while smooth muscle and connective tissue show throughout the closest relationship. Flemming<sup>4</sup> has shown that in the salamander's bladder every possible intermediate stage between the smooth muscle cell and the connective tissue cell can be demonstrated, and all of McGill's observations<sup>6</sup> point to the close relationship existing between smooth muscle and connective tissue.

It is not the part of this article to go exhaustively into the anatomy either of striated or of smooth muscle. I shall confine myself in the main to the question stated above, namely, whether the cells of smooth muscle contain any element comparable to the sarcolemma of striated muscle. But this question cannot be satisfactorily considered without the mention of a number of characteristics, which every histologist has seen, but which nevertheless receive scant consideration in the textbooks and descriptions.

In the first place, then, the fibres in the two tissues, though unfortunately called by the same name, are very far from being the same thing. The fibres of smooth muscle are very much smaller than those of striated muscle. The amphibian *Necturus* is well known to have unusually large cells, but even in this animal the fibres of the intestinal muscle seldom reach a diameter of more than 3  $\mu$ , except at the levels, where they are bulged out by the comparatively huge nuclei. The striated fibres of vertebrates, on the other hand, are rarely less than 10  $\mu$  in diameter, and the average diameter must be set much higher than this, — at from 50 to 100  $\mu$ . It is not uncommon to find in frog's skeletal muscle fibres with a diameter of 200  $\mu$ ; the *Necturus* fibres are, of course, still larger. It may be said, therefore, that the fibres of striated muscle have an average diameter at least 30 times as great as those of smooth muscle, which means, of course, that the cross-sectional area of the former is 900 times greater. So large a difference cannot be left out of consideration even from the anatomical standpoint; it will appear later that it may be of the greatest importance physiologically.

Another very striking difference between the two tissues is in the sharpness with which the fibres stand out from their sur-

<sup>5</sup> EYCLESHYMER: *American journal of anatomy*, 1904, iii, No. 3, p. 285; MCGILL: *Internationale Monatsschrift für Anatomie und Physiologie*, 1907, xxiv, p. 209.

<sup>6</sup> MCGILL: *Loc. cit.*

roundings. The striated fibres are colored deeply by all the common stains, while the spaces between them remain almost or entirely clear. In smooth muscle, on the other hand, it is often quite difficult to distinguish the boundaries of the fibres; these latter stain much less darkly than those of the skeletal muscle, and the spaces between them are filled with a mass of tissue which may, under certain circumstances, be even more deeply stained than the fibres themselves.

These differences, so striking in histological preparations, are in close relation with the results which are obtained by teasing the tissues in the fresh state. Fresh striated muscle may be teased with the greatest readiness into its constituent fibres, while fresh smooth muscle cannot be satisfactorily teased at all; it tears nearly as readily in one direction as in any other. These facts may be justly taken to indicate that the fibres of smooth muscle are firmly bound to one another by a system of interstitial fibrils or membranes.

It has long been stated that the fibres of smooth muscle taper gradually from the region of the nucleus in either direction, but the significance of this fact has not been dwelt upon. The fibres of striated muscle do not have this peculiarity; they remain of the same diameter from one end to the other. The photographs in my previous article<sup>7</sup> show that the striated sarcofibrils are like the fibres in this respect; they maintain the same diameter in all parts of their course through the fibre. The striated fibre, therefore, has all of the peculiarities which indicate that it is a bundle of contractile threads, which run from one end of it to the other without either changing in diameter or branching or ending anywhere in its substance. The tapering of the smooth muscle fibre indicates important differences in the arrangement or character of the elements of which it is composed.

This leads to the consideration of the finer structure of the smooth muscle fibre. Is there any evidence to indicate that it is similar to that of the striated fibre? Such a comparison would, of course, be most satisfactory if fresh tissue could be used as a basis in both cases. But if the results to be obtained from the study of fresh tissue are to be regarded as final, Heidenhain's views must be given up at once. Here the evidence points to the widest possible difference between the two tissues. The fresh fibres of striated muscle always show the most evident signs of regular

<sup>7</sup> MEIGS: *Loc. cit.*

structure, and those of the wing muscles of insects may be teased, while still living, into their constituent elements or sarcostyles. Fresh smooth muscle, on the other hand, cannot be satisfactorily teased at all; it shows little sign of structure of any sort, and the fibre cells often show no sign either of longitudinal or transverse striation.

Very few histologists, however, would be satisfied with the results of such an investigation, and it is therefore necessary to compare also the best possible histological preparations of the two tissues.

There are difficulties in the way even of this comparison. It seems to be a rather general rule that reagents which fix striated muscle without causing it to contract produce a violent contraction in smooth muscle, and *vice versa*. Seventy per cent alcohol, for instance, usually produces little contraction in a fresh frog's sartorius immersed in it; but it causes with great regularity a violent contraction in pieces of the frog's stomach and intestine. Zenker's fluid acts in the opposite way in each case. Saturated corrosive sublimate causes more or less contraction in both striated and smooth muscle. Formaldehyde is disadvantageous for the fixation of any kind of muscle on account of its tendency to swell the tissue.

Confronted by these difficulties, I have adopted the course of using that reagent which causes the least change in the physiological state of the particular tissue to be fixed, — 70 per cent alcohol for the fixation of the striated muscle, and Zenker's fluid for the fixation of the smooth muscle. But I have controlled my results by a comparison of preparations of both forms of tissue fixed in various reagents. It is perfectly possible to obtain examples of both relaxed and contracted striated muscle fixed in 70 per cent alcohol, and of both relaxed and contracted smooth muscle fixed in Zenker's fluid. If a piece of contracted striated muscle fixed in 70 per cent alcohol be compared with a piece fixed in saturated sublimate, the appearance will be seen to be not essentially different in the two cases; the same may be said for a piece of contracted smooth muscle fixed in Zenker's fluid and a piece fixed in 70 per cent alcohol. In other words, the various fixatives alter the appearance of the tissue fixed in them chiefly according as they alter its physiological state; in other respects they all produce about the same effect. Pieces of striated muscle fixed uncontracted in 70 per cent

alcohol may therefore be considered to be very nearly comparable to pieces of smooth muscle fixed uncontracted in Zenker's fluid.

Such preparations I have stained with Heidenhain's iron hematoxylin and with Mallory's phosphotungstic acid hematoxylin<sup>8</sup> and compared by direct microscopic observation. Staining, however, always adds another complicated factor to the changes produced by histological treatment, and I have therefore had unstained sections of striated muscle and smooth muscle photographed by ultra-violet light. Dr. S. B. Wolbach has kindly made the photographs of smooth muscle for me, and I take this opportunity of offering him my sincere thanks. The illustrations of striated muscle are reproductions of some of those which were published in my former article in the *Zeitschrift für allgemeine Physiologie*.<sup>9</sup>

Fig. 1 is reproduced from a photograph by ultra-violet light of an unstained longitudinal section of the uncontracted smooth muscle of the frog's intestine, and Fig. 2 is a similar reproduction from a section of the uncontracted frog's sartorius. In both cases the sections are about  $1\ \mu$  thick, and the amplification is 1800 diameters. In both cases also the treatment of the tissue was the same except for the manner of fixation. The embedding material was of course paraffin. The sections were finally preserved in glycerin and photographed in that fluid. Fig. 1 shows three nuclei and parts of several cells. As will be explained later, however, it is difficult or impossible to make out the cell boundaries in preparations of uncontracted smooth muscle. Fig. 2 shows only a part of one fibre, and no nuclei.

A glance at the photographs reveals the difference in the character of the two tissues better than could pages of written description. While the fibres of the striated muscle are evidently made up of definite elements which have a definite structure and arrangement, those of the smooth muscle show nothing but an extremely irregular arrangement of lines and dots. It may perhaps be said that the lines have a generally longitudinal direction, but beyond this the smooth muscle cells show little more sign of inner structure than does any piece of coagulated protoplasm. Indeed, the appearance of Fig. 1 reminds one strongly of the appearances which Fischer<sup>10</sup> has obtained and described in precipitated hemoglobin and coagulated blood serum.

<sup>8</sup> MALLORY: *Journal of medical research*, 1905, xiii, p. 116.

<sup>9</sup> MEIGS: *Loc. cit.*

<sup>10</sup> FISCHER: *Fixirung, Färbung und Bau des Protoplasmas*, Jena, 1899.

The appearances of cross sections of the two forms of muscle are quite as strikingly different as are those of the longitudinal sections. Figs. 3 and 4 are reproductions of photographs by ultra-violet light of cross sections, exactly similar to the longitudinal sections of Figs. 1 and 2, except for the difference in the direction of the cutting. Here, again, it will be noted that there is nothing in the smooth muscle to correspond to the sharp contrast between the sarcostyles and the sarcoplasm of the striated muscle.

The direct microscopic study of stained preparations adds little to the evidence for Heidenhain's view that the cells of smooth and striated muscle have an essentially similar structure. Sections of uncontracted smooth muscle stained with Heidenhain's iron hematoxylin or Mallory's phosphotungstic acid hematoxylin show nothing to correspond to the sharp contrast between the staining of the sarcostyles and that of the sarcoplasm in striated muscle. In uncontracted striated muscle stained by Heidenhain's method the sarcostyles may appear quite black and opaque, while the sarcoplasm remains practically unstained; whereas longitudinal sections of smooth muscle show only a fine, irregular longitudinal striation exactly corresponding in character to that shown in the photograph by ultra-violet light. The contrast between the more deeply and less deeply stained parts of the cells is never as sharp as in the case of striated muscle, and is often not to be seen at all. Fig. 5 is a photograph by ordinary light of a longitudinal section  $2\ \mu$  thick of the circular muscle of the frog's intestine stained by Mallory's method. The amplification is 1000 diameters.

It is hardly necessary to discuss the question whether the appearance of the longitudinal striations of the smooth muscle cells is or is not an "artefact." Evidence has already been given to show that if these striations are to be taken as the expression of the existence of "fibrillæ," the fibrillæ in question must be admitted to be of an entirely different order from the "fibrillæ," or sarcostyles, of striated muscle. It may be added that all the indications by which it has been laboriously shown that the fibrillæ "pre-exist" in striated muscle, fail in the case of smooth muscle. One form of striated muscle may be teased, while still living, into its constituent fibrillæ; no such form of smooth muscle is known. In all forms of striated muscle the living fibres appear plainly longitudinally striated; the fresh smooth muscle fibres often show no sign of any form of striation. A very small amount of histo-

FIGURE 1. — Longitudinal section of the circular muscular coat of the frog's intestine. The section is one  $\mu$  thick and the tissue was fixed in Zenker's fluid. *a. a.* nuclei. Amplification 1800 diameters. Photographed by ultra-violet light.

FIGURE 2. — Longitudinal section of the uncontracted frog's muscle (sartorius) one and one-fourth  $\mu$  thick. At *a. a.* the characteristic cross striation of the individual sarco-styles can be plainly seen. At *c. c. c.* are seen the remains of membranes which in the living state bind the sarco-styles together. Tissue was fixed in 70 per cent alcohol. Amplification 1800 diameters. Photographed by ultra-violet light.

FIGURE 3. — Cross section of the uncontracted circular muscular coat of the frog's intestine. The section is one  $\mu$  thick and the tissue was fixed in Zenker's fluid. *a. a.* nuclei. Amplification 1800 diameters. Photographed by ultra-violet light.

FIGURE 4. — Cross section of the uncontracted frog's muscle (sartorius) one and one-fourth  $\mu$  thick. Tissue was fixed in 70 per cent alcohol. Amplification 1800 diameters. Photographed by ultra-violet light.

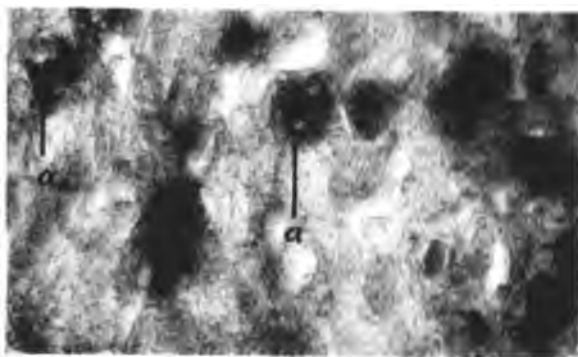
FIGURE 5. — From a photograph by ordinary light of a stained longitudinal section of the circular coat of the frog's intestine. The section was two  $\mu$  thick, was stained by Mallory's method, and photographed in Canada balsam. *a. a.* nuclei. Amplification 1000 diameters. Zenker's fixation.



1



2.



3.



4.



5.

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that shown in Figs. 6 and 7. The condition of the tissue of Fig. 8 corresponds to that of Fig. 6; the same holds true for Figs. 7 and 9.

The condition shown in Figs. 6 and 8 will for the present be called *A*; that of Figs. 7 and 9, *B*. In condition *A* the cells or fibres are rather closely crowded together and stain somewhat less darkly than their nuclei; in longitudinal section they show a fine longitudinal striation, and in cross section a rather irregular arrangement of indistinct lines and patches. The fibres in condition *B* are much more widely separated from each other; they tend to stain solidly and as dark, or darker, than their nuclei. In condition *A* the intercellular tissue appears closer, and it is often rather difficult to make out the borders of the cells; the nuclei are longer, and the cells are not very markedly bulged out in their neighborhood. In condition *B*, on the other hand, the cells are often very much bulged out in the neighborhood of the nuclei, while the diameters of those parts of the cells which lie beyond the nuclear regions are about the same as in the case of condition *A*. May it be supposed that conditions *A* and *B* represent different physiological states of the muscle?

The question of the different forms assumed by smooth muscle at rest and in contraction has been considered by Henneberg<sup>11</sup> and by McGill.<sup>12</sup> These two authors are agreed only in the opinion that there is a difference between contracted and uncontracted smooth muscle; in regard to the appearance of the muscle in each of the physiological states mentioned the two authors are diametrically opposed to each other. Henneberg states that the contracted muscle cells stain less deeply and show a more marked fibrillation, while McGill thinks that both these points are characteristic of uncontracted cells. In neither case is the argument by which it is shown that one type of cell is contracted and the other extended very convincing. Henneberg gives a long description of his methods for obtaining muscle in contraction and relaxation, but ends by saying that both kinds of cells were observed in all the specimens. He does not even clearly state that the form of cell supposed to represent uncontracted muscle is markedly more plentiful in the specimens of the tissue which he endeavored to fix in the resting state. In various other ways the

<sup>11</sup> HENNEBERG: *Anatomische Hefte von Merkel und Bonnet*, 1901, lv, p. 427.

<sup>12</sup> MCGILL: *Anatomischer Anzeiger*, 1907, xxx, p. 426.

article is somewhat unsatisfactory. The author states that he made use of a number of methods in fixing the tissue, and finally found heat to be the best; he used this method to fix his tissue in relaxation. He does not say, however, whether he was careful, in making his comparisons, to use relaxed and contracted tissue fixed by the same method. Finally, there is reason to believe that heat produces a violent contraction in smooth muscle analogous to the heat rigor of striated muscle.<sup>18</sup>

McGill has studied longitudinal sections of cells showing thickenings, and finds that the thickenings show the characters attributed by Henneberg to uncontracted cells. She assumes that the thickenings represent areas of contraction.

In order to discuss this question intelligently, one should have the most definite possible knowledge of the action of fixing reagents on smooth muscle. I have therefore arranged pieces from the frog's intestine so that they could write their contractions on a revolving drum while they were immersed in various fixing fluids.

If a ring be cut from the frog's intestine and so arranged that the contractions of the circular muscle are recorded on a revolving drum, it will be found that the immersion of the piece of muscle in 70 per cent alcohol produces a violent contraction and fixes the muscle in contraction. Zenker's fluid, on the other hand, fixes the muscle at the length it happens to have when the fluid is applied. I have therefore used the latter fixative in my endeavor to discover the differences between histological preparations of contracted and uncontracted smooth muscle.

A difficulty in the way of the further study of this subject is the fact that smooth muscle is capable of maintaining indefinitely a state of marked contraction or tone. It is necessary, therefore, to obtain some criterion by which it may be decided whether any given piece of smooth muscle is relaxed or contracted. This criterion is fortunately given by the behavior of short cylinders cut from the intestine of freshly killed vertebrates. Such cylinders usually take the form shown in Fig. 10; the circular muscle contracts sharply near the cut regions, while that further away from these regions remains more or less relaxed. Such pieces thrown immediately into Zenker's fluid maintain the form represented. Here, at any rate, is the opportunity to compare specimens of more and less contracted smooth muscle.

<sup>18</sup> VERNON: *Journal of physiology*, 1899, xxiv, p. 239.

It is a little difficult to get accurate cross sections of the circular coat, especially in pieces of tissue having the somewhat complicated form shown in Fig. 10. By careful cutting of the tissue before embedding, however, and careful orientation of the embedded tissue, very satisfactory results may often be obtained. Figs. 6 and 7 are different fields from a section of such a piece of tissue, Fig. 7 being from the contracted portion of the circular coat, and Fig. 6 from the uncontracted portion.

I have made a large number of such sections from pieces of the intestine of the frog, *Necturus*, and guinea pig, and have constantly obtained results like those shown in Figs. 6 and 7. The cells of the contracted tissue stain more darkly, show less sign of fibrillation, and lie further apart from each other than those of the uncontracted tissue. My results are therefore in accord with those of McGill and opposed to those of Henneberg.

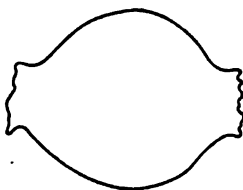


FIGURE 10. — Form taken by short pieces cut off from the intestine of freshly killed animals.

That McGill has failed to observe what is perhaps the most interesting part of the appearance, namely, the change in the relation between the volume of the cells and that of the intercellular tissue, is to be explained from the circumstances that she has observed only nodes of contraction, and has therefore not been able to pay much attention to cross sections. Henneberg's view that the less deeply staining cells are the contracted ones is to be explained from the very interesting circumstance that the contracted cells of smooth muscle have a cross-sectional area very little, if at all, greater than that of the uncontracted cells. This peculiarity of smooth muscle is mentioned by Heiderich;<sup>14</sup> it explains the disagreement between Henneberg and McGill and is confirmed by my own experiments.

The observations which have been reported concerning the circular muscular coat of the small intestine may be repeated with the longitudinal coat. If pieces be cut from the living small intestine of a frog and closely watched, they will usually be seen to shorten markedly immediately after the cutting and to remain shortened. Such pieces hardened in Zenker's fluid and cut into sections show the longitudinal muscular coat in a condition corresponding to that of Figs. 7 and 9. If, however, such pieces

<sup>14</sup> HEIDERICH: *Anatomischer Anzeiger*, 1901, xx, p. 192.

be subjected to the action of ether vapor, the longitudinal muscular coat often relaxes markedly. If such a piece be immediately fixed in Zenker's fluid, sections cut from it will show the longitudinal coat in a condition approaching that of Figs. 6 and 8.

The observations which have just been reported make it probable that the contraction of smooth muscle is accompanied by a passage of fluid from the fibres to the interstitial spaces. The fibres of the contracted muscle, though often less than half as long as those of the uncontracted muscle, have nevertheless almost the same cross-sectional area; the conclusion follows that the contracted fibres have lost considerably in volume. The area of the interstitial spaces is very much greater in the sections of the contracted tissue, and it is just to infer that the substance lost by the cells has passed to the interstitial spaces. The staining reactions confirm still further the conclusions which have been drawn. It is the general rule that those tissues which contain the most solid matter stain most darkly, and this explains the fact that the cells of the contracted muscle, which have lost fluid, stain more darkly than those of the uncontracted muscle.

The behavior of the nuclei also is interesting. It has been said that the nuclei of the contracted tissue are much shorter and thicker, and that the cells are more bulged out at their levels. When it is added that the nuclei of the contracted muscle stain little if at all darker than those of the uncontracted muscle, it will be clear that these bodies take no part in the fluid interchange carried on by the other parts of the cells. The nuclei, in all probability, maintain the same volume through all stages of contraction and relaxation; and hence it is that the nuclei of the contracted cells are markedly thicker, that the cells are more bulged out at their levels, and that there is little difference between the staining of the nuclei of contracted and uncontracted cells.

It need hardly be added that the smooth muscle cells show nothing in the least similar to the characteristic changes which the sarcostyles of striated muscle undergo during their contraction. It has already been said that the longitudinal striations, which have been taken to indicate the existence of fibrillæ in the smooth muscle cells, are so irregular as to make their true significance extremely doubtful. At no stage of contraction or relaxation does the smooth muscle cell show anything approaching the characteristic cross striæ of skeletal muscle.

The study of smooth muscle in contraction, therefore, still further emphasizes the very wide difference between it and striated muscle. It indicates that the contraction of smooth muscle is accompanied by a passage of fluid from the cells to the interstitial spaces; while a similar study of striated muscle indicates that its contraction is accompanied by a passage of fluid from the sarcoplasmic spaces into the sarcostyles.<sup>15</sup> Further, there is no evidence to show that any part of the smooth muscle cell possesses a mechanism similar to that of the striated sarcostyles.

The single point of resemblance, therefore, between striated and smooth muscle seems to be that in both cases contraction is the result of, or at least is accompanied by, a passage of fluid from one part of the tissue to another. The mechanism by which the energy of this flow of fluid is converted into shortening seems to be diametrically different in the two cases; in striated muscle the contractile elements are distended during contraction, while in smooth muscle they are deprived of fluid. In the succeeding section evidence will be adduced to show that artificial swelling, or artificial abstraction of fluid by such means as distilled water, acids, hypertonic salt solutions and drying, produce in both tissues the results to be expected from a consideration of the histological facts which have been reported.

## II. THE CHARACTERISTIC REACTIONS OF SMOOTH MUSCLE AND CROSS-STRIATED MUSCLE TO SWELLING REAGENTS AND TO REAGENTS WHICH WITHDRAW WATER FROM THEM.

This section is to deal with the effects on muscle of swelling reagents and their opposites. I fully realize that in attempting to draw conclusions from these effects I am entering a region of great doubt and difficulty. The recent highly interesting experiments of R. S. Lillie<sup>16</sup> and of Fischer and Moore<sup>17</sup> make probable the view that the swelling of muscle in distilled water is a more complicated phenomenon than has sometimes been supposed. It may very well be that what is ordinarily known as osmosis plays only a minor part in this phenomenon, and that the major part is played by what Fischer has called "the affinity of colloids for

<sup>15</sup> MEIGS: *Loc. cit.*

<sup>16</sup> LILLIE: This journal, 1907, xx, p. 127.

<sup>17</sup> FISCHER and MOORE: *Ibid.*, 1907, xx, p. 330.

water." In the succeeding report of experiments no attempt is made to throw light on this phase of the subject; it is only sought to show that, as a general rule, swelling produces certain effects on muscle, and that abstraction of water from the tissue tends to produce opposite effects.

In weighing the results to be reported, still other considerations must be kept in mind. In the first place, all such reagents as distilled water and hypertonic salt solutions are destructive to muscle, provided they are allowed to act long enough. A frog's sartorius immersed in a 0.2 per cent solution of sodium chloride continues to gain in weight for only about half an hour;<sup>18</sup> it then begins to lose the water it has already absorbed and at the same time, of course, its power to absorb water from hypotonic solutions. There can be no doubt that the loss of this power is the expression of great physical changes in the structure of the muscle, and such changes are exactly what one should expect. The various parts of a muscle immersed in such a reagent as distilled water or 0.2 per cent salt solution must be subjected to very considerable internal pressures, and that these pressures combined with the accompanying diffusion of the muscle salts should be rapidly destructive is not at all a surprising result.

Further, reagents, such as distilled water, affect at first only the outer layers of muscular elements, causing in them a tendency to contract which must overcome the passivity of the still unaffected inner layers. This can only result in still greater injury to many of the elements, and furnishes a ready explanation of the feebleness of the contractions produced in this way. On account of the feebleness of the contractions in question, the muscles must be lightly weighted when they are arranged to record their contractions on a revolving drum.

Lastly, reagents, such as distilled water and hypertonic salt solutions, often act as stimulants to irritable muscle immersed in them. The first effect of immersion is often to produce twitchings or single contractions and relaxations in the muscle, which in rapidity and other respects resemble the contractions produced by electric stimulation or by the nerve impulse. These contractions are, however, quite different from the slow contractions and relaxations which are peculiarly the effect of swelling or abstraction

<sup>18</sup> FLETCHER : *Journal of physiology*, 1904, xxx, p. 423, Fig. 5.

of water from the muscle. The two classes of phenomena must be sharply distinguished from one another.

A fresh frog's sartorius placed in distilled water becomes more and more swollen and at the same time gradually shortens. Under favorable circumstances the shortening may amount to about 45 per cent of the original length of the muscle. Fig. 11 is a fairly typical record of such a "water rigor" in the frog's sartorius.

Regarding this and the subsequent records, the following remarks may be made: The arrows, showing the points at which the various reagents were applied, were drawn in free-hand, and

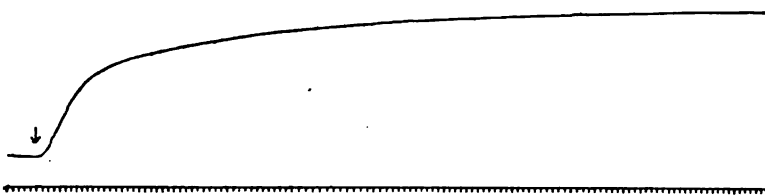


FIGURE 11. — One fourth the original size. Curve showing the course of water rigor carried nearly to its completion in a frog's sartorius. The arrow marks the point at which the distilled water was applied. The muscle had a length of 25 mm. at the beginning of the experiment, and was weighted with 0.6 g. Magnification of writing point, 4.77; proportional contraction, 45 per cent. The time is marked in minutes.

are therefore only approximately accurately placed. The first results of the application of the reagents usually appear in from fifteen to thirty seconds. The slight irregularities to be observed in most of the curves are the results of jarring, unfortunately not to be avoided in records requiring such long periods of time and made in a city laboratory. In all the records the actual contraction of the muscle is magnified 4.77 times by the arrangement of the writing lever, but the length of muscle between its attachments was much less in the case of the smooth muscle curves. A given rise or fall in the curve represents, therefore, a much greater percentage of shortening or lengthening in the case of the smooth muscle. In all cases a rise in the curve represents a shortening of the muscle, and in all cases the muscle was weighted with six-tenths of a gram. The smooth muscle records were obtained with rings cut from the frog's stomach. The rings in question had a breadth (in the longitudinal direction of the stomach) of 2 or 3 mm., and a thickness of about 1 mm. It was found necessary to tear the mucous lining out and use only the muscle ring in ob-

taining the record; otherwise the swelling of the mucous membrane in the distilled water masks the lengthening of the muscle.

Fig. 12 represents the effect of replacing the distilled water with a 2 per cent solution of sodium chloride at an early stage of water rigor; and Fig. 13, the effect of using, instead, a 0.7 per cent solution. As the records show, the contraction is rapidly

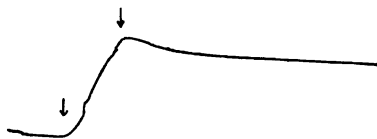


FIGURE 12. — Two thirds the original size. Curve showing a partial water rigor and its reversal in a frog's sartorius. The first arrow marks the point at which the muscle was immersed in distilled water; the second, that at which the distilled water was removed and 2 per cent sodium chloride solution applied. The time is marked in minutes.

brought to a close, and a slow lengthening of the muscle begins.

That the 0.7 per cent solution acts more effectively in removing the water rigor contraction than the 2 per cent solution is a fact of great interest, but the analysis of this and of a number of other points must be left for a later article.

Fig. 13 is interesting because it shows a phase of water rigor absent in the other two curves, but nevertheless quite common. It frequently happens that the applica-

tion of the distilled water is not immediately followed by the slow shortening. A period of two or three minutes often intervenes in which the muscle shortens very little or not at all, but shows an increase of irritability marked by numerous rapid twitches. This period is, however, as in Fig. 13, always soon succeeded by the slow and even shortening characteristic of water rigor.

The phenomena which have just been described are absolutely characteristic of striated muscle. Pieces of fresh skin, nerve, and tendon from the body of the frog immersed in distilled water, or in a hypertonic salt solution, absorb the former and give up water to the latter. But the changes in form which they undergo are quite different from those which have just been described for muscle. They all increase in all dimensions under the influence of the swelling, and decrease in all directions under the influence of the shrinkage. In the nerve and tendon the changes in volume take place chiefly in the transverse dimensions. The changes in length in pieces of nerve and tendon immersed for three hours and a half in distilled water were not more than 1 per cent of the original length. A piece of skin immersed for the same period



in distilled water increased about 10 per cent in each dimension in the plane of the surface of the body from which it was cut.

Pieces of smooth muscle immersed in distilled water, or in 2 per cent salt solution, exhibit phenomena which are quite as characteristic for smooth muscle as are those which have been described above for striated muscle, but which are almost exactly the opposite of the phenomena described for striated muscle. Two

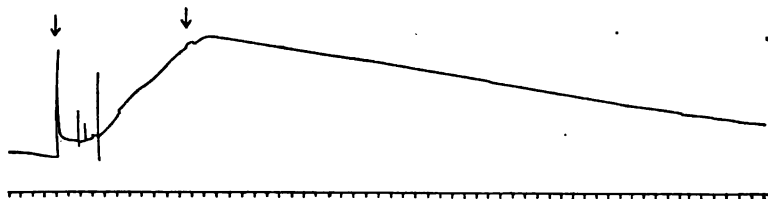


FIGURE 13. — Four fifths the original size. Curve showing a partial water rigor and its reversal in a frog's sartorius. The first arrow marks the point at which the muscle was immersed in distilled water; the second, that at which the distilled water was removed and 0.7 per cent sodium chloride solution applied. The time is marked in minutes.

rings of the same size were cut from a frog's stomach and immersed at the same time, one in distilled water and the other in 2 per cent salt solution. At the end of half an hour the first had a diameter two and one-half times as great as the second. The difference in the thickness of the muscular layer in the two cases was much less marked than the difference in length.

Except that they occur in the opposite direction, the contractions and relaxations of the smooth muscle bear a strong resemblance to those of the striated muscle. They occur perhaps somewhat more feebly in the smooth muscle, but they have the same gradual, even character. In both cases the process may be reversed and re-reversed several times in the same piece of muscle, and in both cases the process is slower and shows less tendency to complete itself after each reversal. In both cases the extreme amount of contraction and relaxation which can be produced in this way is about the same as that which occurs in the strongest tetanus.

To avoid all possible doubt as to the reaction's being really that of smooth muscle, I have torn out the mucous lining from rings of the frog's stomach and compared the behavior of the mucous rings with that of the rings of muscular tissue. The muscular rings behave exactly the same whether they have the mucous lining

or not, except that the mucous lining filling the lumen of the contracted ring prevents it from contracting to its fullest extent. The mucous rings show less change in diameter than the muscle rings; and the change which they do show is due chiefly to the fact that the mucous membrane swells enormously in every dimension when immersed in distilled water. The contraction of the smooth muscle, like that of the striated, is feeble, but nevertheless capable of lifting small weights; it may therefore easily be recorded.

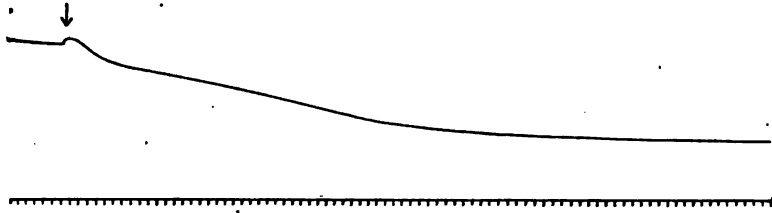


FIGURE 14. — Four sevenths the original size. Record of the effect of distilled water on the smooth muscle of the frog's stomach. The arrow marks the point at which the distilled water was applied. At the beginning of the experiment the muscle had a length of 8 mm. between its attachments; and at the end, a length of 13 mm. The lengthening was therefore 62 per cent of the original length. Magnification of writing lever, 4.77; muscle weighted with 0.6 gm.; time marked in minutes.

Fig. 14 represents the behavior of a ring from the frog's stomach immersed in distilled water for a period of one hour and twenty minutes. The first effect of immersing the muscle in distilled water is to produce a single contraction in all respects like the contraction produced by stimulating the muscle with a single electric shock. After executing this single contraction the muscle lengthens slowly until it reaches a maximum length which it maintains.

Figs. 15 and 16 represent the effect of replacing the distilled water by a 2 per cent solution of sodium chloride at an earlier and at a later stage of the relaxation. In both cases the application of the hypertonic solution is followed by a preliminary lengthening, which is succeeded by a more marked shortening. The preliminary lengthening is less marked, and the succeeding shortening more marked, the earlier the stage at which the hypertonic solution is applied. If the original lengthening under the influence of distilled water be carried to an extreme degree, the application of the hypertonic salt solution may be followed by lengthening without any subsequent shortening.

It is a general rule that swelling reagents produce shortening of striated muscle and lengthening of smooth muscle, and that abstraction of water has in both cases the opposite effect. Moderately weak acids are known to cause the swelling of muscle immersed in them, and they produce changes in length in both striated and smooth muscle analogous to the changes produced by immersion in distilled water, though more rapid. Drying, on the other hand, by which of course water is removed from the muscle,

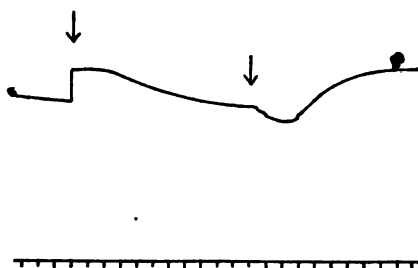


FIGURE 15. — Original size. Record of the effects of distilled water and 2 per cent sodium chloride solution on the smooth muscle of the frog's stomach. The first arrow marks the point of application of the distilled water; the second, that of the salt solution. Magnification of writing lever, 4.77; length of muscle between attachments at end of experiment, 5 mm.; time marked in minutes.

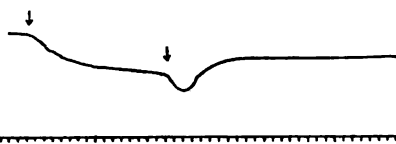


FIGURE 16. — Two thirds the original size. Record of the effects of distilled water and 2 per cent sodium chloride solution on the smooth muscle of the frog's stomach. The first arrow marks the point of application of the distilled water; the second, that of the salt solution. Magnification of writing lever, 4.77; length of muscle between attachments at end of experiment, 5 mm.; time marked in minutes.

produces a very slow contraction in smooth muscle, and, as a rule, a slight lengthening in striated muscle. In the case of the striated muscle the lengthening may be considerable if the muscle be first caused to shorten by immersion in distilled water. I have tried the drying experiments simply by allowing the muscles to dry in the air of the laboratory without artificial air currents; under these circumstances the reactions are much slower than those produced by the hypertonic salt solutions.

To the rule which has just been stated there are a number of exceptions, and some of these must now be briefly discussed. If the irritable striated muscle of a frog be immersed in concentrated glycerin, it almost immediately goes into a form of maintained contraction more or less resembling the rigor produced by chloro-

form. But this contraction is much more rapid than the contractions and lengthenings caused by distilled water and hypertonic solutions, and must be regarded as merely an apparent exception to the general rule.

The results of immersing striated muscle in sodium chloride solutions having strengths of from 0.3 per cent to 0.7 per cent are perhaps somewhat more difficult to explain. Such solutions cause considerable swelling of the muscle immersed in them, but no shortening. In attempting to interpret these facts, however, it is necessary to remember that the muscle is not simply a solution with a given osmotic pressure surrounded by a semi-permeable membrane. Any fluid passing into the muscle must enter first the spaces between its fibres, then the sarcoplasmic spaces within the fibres, and, last of all, the sarcostyles. It is highly probable that a resistance to its course is offered by the sarcolemmas of the fibres and again at the surfaces of the sarcostyles; and whatever views may be held as to the nature of water rigor, the most probable explanation of its non-occurrence in the case of hypertonic sodium chloride solutions above 0.3 per cent is that these solutions fail to penetrate the peculiarly contractile elements.

The main object of the present article is to point out the relation which exists between the results reported in the first section and those reported in the second section. Histological studies of relaxed and contracted striated muscle indicate that contraction is, in this form of muscle, accompanied by a swelling of the contractile elements. In harmony with this, it is shown that, as a rule, swelling reagents cause a slow contraction in this form of muscle, and that the opposite class of reagents cause a slow lengthening. Histological studies of relaxed and contracted smooth muscle, on the other hand, indicate that the contraction of smooth muscle is accompanied by a loss of fluid on the part of its contractile elements. And in the case of smooth muscle swelling reagents usually produce a slow lengthening, and the opposite class of reagents, a slow shortening. The inference toward which these observations point is that in both classes of muscle contraction is normally the direct mechanical result of the passage of fluid from one part of the tissue to the other.

In considering the exceptions to the rule which I have sought to establish in the second part of the article, it must be remembered that the inference which has just been stated does not make

contraction or relaxation depend on changes in the volume of the muscle as a whole, but merely on changes in the volume of the contractile elements. The fact, therefore, that the whole muscle may change in volume without changing in length, or may change in length without changing in volume, is obviously not a contradiction of the inference.



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